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CONTAMINANT CONCENTRATIONS IN, AND EFFECTS ON, YELLOW PERCH (*PERCA FLAVESCENS* (MITCHILL)) AND SPOTTAIL SHINER (*NOTROPIS HUDSONIUS* (CLINTON)) OF THE ST.LAWRENCE RIVER

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CHAPTER 1

ORGANOCHLORINE AND HEAVY METAL RESIDUES IN YOUNG-OF-THE-YEAR FORAGE FISH

CHAPTER 2

BACKBONE AS A TOOL FOR MEASURING SUBLETHAL EFFECTS ON FISH

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2 ABSTRACT

In chapter 1 the results of the organochlorine and heavy metal residues in young-of-the-year spottail shiner and yellow perch are discussed. Within the framework of the Inland Waters Directorate's, Quebec Region, mandate for the study of toxic chemicals in the St.Lawrence River, young-of-the-year fish have been sampled during September 1986 as the continuation of a pilot study which had been carried out in 1984. Its aim is a further evaluation of the use of young-of-the-year fish as a bioindicator for ambient concentrations of organochlorinated contaminants and heavy metals in the water column and their spatial distribution throughout the river.

In the St.Lawrence River spottail shiner (Notropis hudsonius) and yellow perch (Perca flavescens) were found to be the most suitable species, because they are ubiquitous, abundant and easy to identify.

A total of 33 stations in the St.Lawrence River and certain tributaries between Beauharnois and Trois-Rivières have been sampled. In particular, Lake St.Pierre was highlighted. At 19 stations, either spottail shiner or yellow perch were analysed, while at 3 stations it was possible to analyse both species, so a comparison of their bodyburden contents could be made. For both spottail shiner and yellow perch the variation in bodyburden contents of contaminants between replicates at a sampling site were small.

The results show that there are point sources of mercury and HCB in the St.Louis River and a point source of cadmium in the Beauharnois Canal. At 18 of the 22 stations, PCB levels in the fish are above the aquatic health protecting guideline of 0.1 mg/kg fish (wet weight).

Because spottail shiner is not present at every site, yellow perch was also sampled, and the relationships between contaminant concentrations in both species were investigated, based on results from this study and the 1984 pilot study. It was found that yellow perch is a good replacement species for spottail shiner, especially for the organochlorinated contaminants and mercury, and bodyburden contents seem directly comparable. For the other metals, either a conversion factor is necessary, or no correlation was found.

Nickel and chromium were found to be spatially highly correlated, as were selenium and PCB's. The organochlorinated contaminants were correlated with the lipid percentages only in the case of spottail shiner.

Length and lipid differences found between the sampling sites are making the comparison between stations more difficult, since organochlorinated contaminants were found to correlate with length and lipid within a station.

In chapter 2 the result of the backbone analyses for yellow perch are discussed. Backbones were analysed for collagen and hydroxyproline contents, because these concentrations can be altered

through exposure to contaminants. In this study, no differences were found between a reference (clean) and a more polluted station, nor any correlation between pollution and deformities of the backbones (investigated by means of X-rays). The results may have been influenced by the heterogeneity of the batch, but the variation found between replicates within a fish and within a yearclass at one station were quite high, which affects the sensitivity of the tool.

CHAPTER 1

ORGANOCHLORINE AND HEAVY METAL RESIDUES IN YOUNG-OF-THE-YEAR FORAGE FISH

1.1 INTRODUCTION

The following description of the St.Lawrence River is derived from SLOTERDIJK (1987). The St.Lawrence River is one of the major rivers of North America, and flows for about a 1000 km between the Great Lakes and the Atlantic Ocean (figure 1). It is formed by the discharge of Lake Ontario, which is known for its highly contaminated character (NRIAGU & SIMMONS, 1983). A major tributary, the Ottawa River, enters the St.Lawrence just before the Montreal Island, and remains a very distinct water mass with a lower conductivity and higher turbidity (GERMAIN & JANSON,1984). It mixes only partially with the water of the St.Lawrence River, originating from Lake Ontario, thus forming a central intermediate third water mass. The two original water types do not fully mix until Portneuf, 150 km downstream, where the tidal influences causes current reversal. This difference is of great importance, since the Ottawa River transports less contaminants than the water coming from the Great Lakes (Lake Ontario).

The main sources of pollution, which influence the water quality in the St.Lawrence River, are the industries around the Great Lakes in both the United States and Canada, and the industrial activities of the Montreal-Sorel corridor. Indeedm, the Great Lakes Basin, including the international section of the St.Lawrence River and the Montreal-Sorel corridor, is considered to be the industrial heartland of North America.

Since half of the population of the Quebec Province is dependent on the river's water quality for drinking water and recreational activities, it has been studied since the beginning of the seventies (ANONYMOUS, 1978a), although water samples were already taken before that period (JANSON & SLOTERDIJK, 1982).

Because water samples supplied only a part of the information on the presence and behaviour of toxic chemicals in the St.Lawrence, sediments (e.g. JARRY, 1986; SLOTERDIJK, 1984) and fish (e.g. SLOTERDIJK, 1977) have also been analysed. In the latter instance, the muscle tissue of adult fish was analysed to supply information on the bioavailability, bioconcentration and spatial distribution of toxic chemicals in the St.Lawrence River.

The variation, however, in contaminant residues concentrations in adult fish at one sampling site were found to be very large. Therefore, a preliminary study was carried out in 1979 to investigate the possibility of using forage fish as a biomonitoring tool in the St.Lawrence River (GUAY, 1979). Young-of-the-year spottail shiner (Notropis hudsonius (Clinton)) was already being used as an bioindicator for toxic substances in the Great Lakes (SUNS & REES, 1978). Forage fish is a general term used to describe young fish and cyprinids, which form an important food source for larger or older piscivorous fish species. GUAY (1979) found that besides spottail shiner, yellow perch (Perca flavescens (Mitchill)) was present at most sampling sites and could therefore be a replacement species at

stations where the former would be absent. Consequently, both species were chosen as a tool to investigate the bioavailability and spatial distribution of organochlorine residues and heavy metals. An important aspect, besides their omnipresence, is that they are also very easy to identify in the field. In general, minnows (Cyprinidae) are difficult to identify, but spottail shiner has a large black spot on its tail by which distinguishes it from all the other minnows.

The forage fish should be sampled during a period of a few weeks in September at the end of their first growing season, because they have stayed the whole summer at the same site, and have had the maximum time available to accumulate contaminants. The young fish begin to migrate to deeper water in October through which the catchability declines; in the 1984 study there was more effort needed to catch the forage fish in October than in September (GUAY & DANDERAND, 1986). Since it was clear from the 1984 study, that most contaminants are concentrated up to a level well above the detection limit, it is not necessary to postpone the sampling until May of the following year to increase exposure time, before the new yearclass hatches.

In 1984, young-of-the-year spottail shiner and yellow perch were for the first time used as bioindicators of toxic chemicals in the St.Lawrence River (GUAY & DANDERAND, 1986). The results of this pilot study were very encouraging, as it supplied additional and new information on the behaviour of contaminants in the aquatic system; furthermore, variations within one sampling site were considerably smaller than found in adult fish and it was possible to detect significant differences between stations which were located very close to each other.

The present study of the forage fish, carried out in 1986, formed a part of the monitoring program in the St.Lawrence River by Environment Canada. In 1986, the objective was to study Lake St.Pierre in more detail and besides forage fish, adult fish and sediment sampling also took place (HARM SLOTERDIJK, pers.com.). The sampling strategy for forage fish in Lake St.Pierre consisted of sampling upstream, within, and downstream of the lake as well as at the mouth of the most important tributaries. To correlate bodyburden contents in forage fish with sediment contaminant levels was, however, not the main objective of the overall activities in Lake St.Pierre (a river is an open flowing system and sediments are considered not to be directly related to the above water). The south shore of Lake St.Louis was sampled again, because GUAY & DANDERAND (1986) detected elevated levels of HCB, PCB's and mercury in this area. The sewage outfall of the Montreal Urban Community at île Ste.Thérèse was sampled in order to investigate the impact of untreated sewage on the presence and bioaccumulation of toxic chemicals.

1.2 MATERIAL AND METHODS

1.2.1 Sampling Program Of The Forage Fish

1.2.1.1 Selection Of The Stations

Sampling took place between the 2nd and 27th of September, 1986, and was carried out by contract (Environnement Illimitée of Montreal) with field assistance from the author. Some tributaries on the south shore were sampled by Environnement Québec (table 1). A total of 34 stations were selected (figure 2): stations 1 to 4 (Lake St.Louis) were chosen because of high bodyburden contents of PCB's and Hg in 1984 (GUAY & DANDERAND, 1986); sampling of stations 11 to 17 was to investigate the influence of the Montreal Urban Community (MUC) sewage outfall; stations 21 to 35 and 41 to 47 were selected for the spatial trend study on Lake St.Pierre: 21 to 35 represent the stations in the river where 41 to 47 were in the tributaries near the mouth; finally, station 48 was selected to measure the input of the Assomption River into the St.Lawrence on the north shore, just downstream of the Montreal Island. Station 27 was sampled twice, once at the beginning and once at the end of the sampling program, to measure the change in bodyburden content during this period.

Table 1. Station list with sampling date, sampling agency and selection of station and species for chemical analysis. s = seine fishing; e = electrofishing; ss = spottail shiner; yp = yellow perch.

station #	location	sampling date	Environnement Illimitée	Environnement Québec	chemical analysis
1	Melocheville	22-SEP-86	s		ss
2	upstream Union Carbide	22-SEP-86	s		
3	downstream Union Carbide	23-SEP-86	s		ss
4	St.Louis River	24-SEP-86	s		ss
11	upstream l'Île Ste.Thérèse	25-SEP-86	s		ss
12	Île St.Patrice	25-SEP-86	s		ss
13	Île Ste.Thérèse	25-SEP-86	s		ss
14	Île Vert	26-SEP-86	s		ss
15	Île Deslauriers	26-SEP-86	s		ss
16	Repentigny	26-SEP-86	s		ss
17	Varennes	26-SEP-86	s		
21	Île au Dragon	18-SEP-86	s		ss
22	Île St.Ours	12-SEP-86	s		ss
23	Lanoraie	08-SEP-86	s		
24	Les Grèves	09-SEP-86	s		
25	Îles aux Foins (Berthierville)	27-SEP-86	s		ss
26	St.Joseph de Sorel (downstream Tioxide)	10-SEP-86	s		
27	Sorel (downstream Tioxide)	10-SEP-86	s		ss+yp
27	Sorel (downstream Tioxide)	27-SEP-86	s		ss+yp
28	Sorel (Île Ronde)	11-SEP-86	s		yp
29	Sorel (Chenal des Barques)	11-SEP-86	s		yp
30	Berthierville (downstream Île aux Vaches)	15-SEP-86	s		ss+yp
31	Trois-Rivières (North shore)	17-SEP-86	s		yp
32	Trois-Rivières (South shore)	17-SEP-86	s		yp
33	Lake St.Pierre (South shore)	abandonned			
34	Lake St.Pierre (centre)	16-SEP-86	s		
35	Lake St.Pierre (North shore)	16-SEP-86	s		
41	Richelieu River	10-SEP-86	s	s	ss
42	Yamaska River	04-SEP-86		e	yp
43	St.François River	03-SEP-86		e	ss+yp
44	Nicolet River	17-SEP-86	s	s+e	
45	Bayonne River	11-SEP-86	s		
46	Maskinongé River	15-SEP-86	s		yp
47	du Loup River	16-SEP-86	s		
48	Assomption River	10-SEP-86		e	

1.2.1.2 The Fishing Gear

Fishing was carried out with a 30.5 m seine, which had a 2.4 cubic metre bag (codend) with a mesh width of 0.64 centimetre. When the water was deeper than 1 metre, the seine was pulled on the one end by a boat and on the other end by walking. In shallower water both ends were pulled walking.

Besides using a seine, electrical fishing gear was also tried out. Environnement Illimitée used a Smith Root GPP 5.0 for the electrofishing. The electric field of this equipment has a radius of about 5 metres. With the electrofishing a greater area can be screened faster, but fishing with a seine seemed more efficient under the circumstances (low current, high turbidity). Environnement Québec used electrofishing for catching, whereas Environnement Illimitée used the equipment only for localizing concentrations of the fish (table 1).

1.2.1.3 The Sample Composition

To be sure the sample consisted only of young-of-the-year fish, spottail shiners smaller than 60 mm and yellow perch smaller than 80 mm were selected. This selection was made based on the results of scale readings carried out during the 1984 pilot study (GUAY & DANDERAND, 1986).

Since 5 replicates per station for chemical analyses was the objective, the minimum total amount of fish per station needed was 250 g per species. When, however, more fish could be sampled, without much effort, the amount caught per station was increased. This surplus could be used to gain more insight into the forage fish as a biomonitoring tool.

1.2.2 Preparation Of The Samples

After capture, the fish were packed in hexane-rinsed aluminium foil and frozen in coolers on dry ice. In the laboratory random samples of 50 g were taken from the total catch per station (well-mixed) for chemical analysis, and the total length was measured for every fish. For spottail shiners, the total length is a better measure than the fork length, because the tail is very often split (McCANN, 1959).

Table 1 lists the stations for which forage fish were analysed. Per station, 3 to 8 samples were submitted to be analysed. For a few stations, samples were also made up of 0.5 cm interval size groups, to investigate the correlation between length and contamination within one station. For spottail shiner, 4 size groups of between 35 to 55 mm were prepared from fish taken at station 16, while for yellow perch 6 size groups of between 55 to 85 mm were taken at station 28. A total of 115 samples were prepared for chemical analysis, the limiting factor being analytical credits (National Laboratory) and budgetary allowances (private laboratory). Sample selection was optimized to obtain maximal spatial coverage, retain statistical reliability, and obtain some detailed comparative analyses (e.g. species comparison and length-contaminant relationship).

1.2.3 Analysis

1.2.3.1 Homogenization

Before analysis, the samples were homogenized by means of a Polytron homogenizer, type PT 10/35, for 3 to 5 minutes. The homogenization was carried out by Novalab Ltd, a private laboratory. The mixture was separated in 2 equal parts: one part for the analysis of lipids and organic contaminants to be analysed by Novalab itself, and another for heavy metal analysis. The second aliquot was shipped on dry ice to the Canada Centre for Inland Waters in Burlington (Ont.) for analysis by the National Water Quality Laboratory.

1.2.3.2 Lipid Percentages

The lipid percentages in the fish were determined, according to the Fish Inspection Branch method (ANONYMOUS ,1970) as follows: a mixture of fish homogenate, acetone, hexane and anhydrous sodium sulphate was homogenized for 1 minute; an equal amount of anhydrous sodium sulphate was added, mixed with the homogenate, and then filtered; the solvent in the filtrate was evaporated at room temperature and the lipid residue was determined gravimetrically. ANONYMOUS (1985a) gives a more detailed description of the lipid procedure as well as of the organic contaminants analyses in connection with the previous forage fish pilot study in 1984.

1.2.3.3 Organic Contaminants

The analyses of PCB's and organochlorinated pesticides were carried out according to a methodology standardized by the Bureau d'Etudes sur les Substances Toxiques (ANONYMOUS , 1980), as described in a previous analytical report of Novalab (ANONYMOUS, 1985a). The homogenates were extracted, after dehydration with anhydrous sodium sulphate, with a 2:1 hexane-acetone mixture. After clean-up, the separation was carried out by elution on a florisil column, using various ether-hexane mixtures. The extracts were concentrated and analysed on a gas chromatograph coupled with a Ni63 electron-capture detector. In 1986 only the hexane fraction was analysed. From this fraction the concentrations of the organic contaminants Aldrin, Arochlor® 1242, 1254 and 1260, p,p'-DDE, HCB, Heptachlor and Mirex can be determined. In the 1984 study the organic contaminants in the other fractions (6% ether-hexane and 50% ether-hexane) were seldom above detection limit (ANONYMOUS , 1985a) and therefore not analysed in the 1986 study. The contaminants analysed, including the metals, and their detection limits are listed in table 2.

Table 2. Detection limits of the organic compounds and the heavy metals.

organic compound:	detection limit: (ppm)
Aldrin	0.001
p,p'-DDE	0.001
HCB	0.001
Heptachlor	0.001
Mirex	0.001
PCB's	
-Arochlor 1242	0.01
-Arochlor 1254	0.01
-Arochlor 1260	0.01
Metal:	
arsenic	0.05
cadmium	0.02
chromium	0.2
copper	0.2
lead	0.1
mercury	0.01
nickel	0.05
selenium	0.05
zinc	0.05

1.2.3.4 Heavy Metals

The heavy metals were analysed by the National Water Quality Laboratory, Inland Waters Directorate, Canada Centre for Inland Waters, Burlington (Ont.) following the methods described in ANONYMOUS (1986). Mercury concentrations were, after digestion with hydrogen peroxide, sulfuric acid, and oxidation with permanganate and persulfate, determined by flameless cold vapour atomic absorption spectrometry. Cadmium, chromium, copper, nickel, lead and zinc were, after digestion with nitric and sulfuric acid, analysed by flame atomic absorption spectrometry, while arsenic and selenium concentrations were quantified by the ICAP (inductively coupled argon plasma) method. The detection limits are listed in table 2.

1.2.4 Statistics

The data were treated on a Microvax II with the use of RS 1 software package (ANONYMOUS , 1984; ANONYMOUS , 1985b). The basic descriptive statistics were determined and results were tested by means of ANOVA, linear regression and Pearson's and Spearman's

correlation (SOKAL & ROHLF, 1981).

1.3 RESULTS

1.3.1 Catch Results

The fish species which were caught during this study are listed in appendix A.1; the nomenclature is according to SCOTT & CROSSMAN (1973). These authors include our study area within the distribution of spottail shiner and yellow perch in the St. Lawrence River. Nevertheless, they were not found at every station, probably due to specific habitat requirements.

In practice the yield of the fishing can be influenced by 2 factors: 1. Absence or low density of the desired species. 2. Obstacles (e.g. deepness, vegetation, rocks, current) which reduce fishing efficiency: too deep water gives the fish the opportunity to dive under the seine; too much vegetation causes the seine to roll up and to pass too close to the surface; rocks prevent or impair the continuous movement of the seine, and a strong current gives difficulties in obtaining and maintaining the efficient half-moon shape of the seine in the water; due to the small mesh size, it was at certain stations, impossible to pull the seine upstream. A detailed description of the conditions at the time of fishing is giving by GUAY (1986).

Sometimes, it was impossible to catch enough fish at a certain station. In such case, attemps were made to catch the fish somewhat more up- or downstream of the station, after it had been verified that no point sources were present between these sites. Consequently, station 23 was replaced more upstream by station 21, and station 24 more downstream by station 25. This approach was, however, not applicable for some tributaries on the north shore of Lake St.Pierre. Therefore the information available for the north shore tributaries is a rather scarce.

Only in four cases, at station 30, 43 and twice at station 27, both species were caught in sufficient quantity so that comparison of the bodyburden contents in the two species was possible.

Contrary to the 1984 pilot study (GUAY & DANDERAND, 1986), hardly any young-of-the-year yellow perch were caught upstream of Sorel (station 27), and almost no young-of-the-year spottail shiners were caught, except for the tributaries, downstream of station 27. In 1984, spottail shiners were caught down to Portneuf, which is halfway between Trois-Rivières and Québec (figure 1). It may be that, since Trois-Rivières is described as the limit of its distribution area (SCOTT & CROSSMAN, 1973), its presence somewhat downstream of Trois-Rivières is not constant from year to year.

At stations, where no spottail shiner or yellow perch were analysed, the yield of these two species was too low to make replicate samples (table 1).

At a few stations, other species such as white sucker (Catostomus commersoni), emerald shiner (Notropis atherinoides) and golden shiner (Notemigonus crysoleucas), were sampled, but, until now, have not been analysed. Older spottail shiner and yellow perch were also collected, and have been retained for possible analysis of toxic effects, using the backbone for frequency of deformities, protein content and mechanical strength (see also chapter 2 of this report).

1.3.2 Bodyburden Contents Of Contaminants In Forage Fish

The concentrations of organic contaminants and heavy metals in spottail shiner and yellow perch are presented as bar histograms in the figures 3 to 14. The bars represent the mean and the error bars the standard error of the mean. The mean, standard error of the mean, and the count per station are listed in appendix A.2. To include the individual measurements below detection limit in the calculation of the mean, a value half of the detection limit was taken. If a mean of a station was below detection limit, a bar half the height of the detection limit is presented without error bars. In principal it would be better to take the median of a station when values below detection limit are involved (H.SLOTERDIJK pers.com.), but this gave some difficulties with computations. Statistical comparisons should only be made between means which are above the detection limit. It cannot be stated that values below detection limits are identical, even if they have been presented as such in the histograms.

1.3.2.1 Organochlorinated Compounds

The results of Aldrin,Heptachlor and Mirex are not presented in figure-form. Mirex was not detected, and Aldrin and Heptachlor were found only at station 16.

DDE is omnipresent (figure 3); the main source seems to be the Great Lakes, because the highest values are found in the central watermass of the St.Lawrence River (stations 11 to 15). The concentrations are, however, far below the aquatic health guideline of 1 mg DDE / kg fish (wet weight) (ANONYMOUS, 1978b).

HCB concentrations are often below detection limit but there is, however, one point source from the St.Louis River (figure 4, station 4), probably due to the chlor-alkali plant present there.

Like DDE, PCB's are omnipresent at the investigated stations, but at much higher levels (figure 5). The concentrations fluctuate somewhat in the river with the higher values in the central watermass

(stations 11 to 14). From the entrance of Lake St.Pierre (stations 25 to 30) to the exit (stations 31, 32), the concentrations in the fish diminish. The tributaries of the St.Lawrence River around Lake St.Pierre may present a dilution factor as the PCB levels in the fish from these tributaries (stations 41 to 46) are generaly lower than those in the river itself. Especially along the shore, where most of the sampling took place, the influence of the tributaries could be significant. Of the 22 stations sampled, 18 were significantly above the aquatic health guideline of 0.1 mg PCB / kg fish (wet weight) and of the other 4 only 2 were significantly below this aquatic health guideline (ANONYMOUS, 1978b).

1.3.2.2 Heavy Metals And Metalloids

Mercury concentrations are at all stations (figure 6) well below the aquatic health guideline of 0.5 mg Hg / kg fish (wet weight) (ANONYMOUS, 1978b). There is, however, a point source in the St.Louis River (station 4). It is suspected that HCB and mercury have the same source, Industries PCG Canada ltee. This chlor-alkali plant uses a mercury electrode and discharges 57.7% of the known industrial input of mercury in the St.Lawrence River between Cornwall and Sorel (ANONYMOUS, 1983). Since 1972 mercury emissions from chlor-alkali plants are regulated by the amount of chlor produced (maximal emission: 2.5 mg Hg/kg Cl2 (ANONYMOUS, 1983)) and the emissions have dropped considerably since then from 12 kg of mercury to about 0.5 kg/day (SLOTERDIJK, 1979).

Mercury residues in piscivorous fish such as walleye (Stizostedion vitreum) and pike (Esox lucius) of the St.Lawrence River have been found to be higher than in any other fish (CHAMPOUX & SLOTERDIJK, in press; GOULET & LALIBERTÉ, 1982b, LÉVESQUE & POMERLEAU, 1986, SLOTERDIJK, 1977). Thus, even these values of mercury below the guideline may still be indicative of a potential problem through foodchain accumulation, as is still found to be the case in southern Lake St.Louis (downstream from station 4), where most adult piscivorous fish were above 0.5 mg/kg (CHAMPOUX & SLOTERDIJK, in press, SLOTERDIJK, 1977).

Cadmium levels are often below detection limit and it is difficult to discover any spatial patterns (figure 7). The higher value at station 3 could have as source the Canadian Electric Zinc Ltd., situated along the Beauharnois canal, which discharges 14 kg of cadmium per day (ANONYMOUS , 1983), as no increased levels of the cadmium are found at station 1. This result is in accordance with the spatial patterns found in 1984, when spottail shiners were sampled just upstream, in Lake St.Francis, and downstream of the Beauharnois Canal. In the former, the cadmium levels in spottail shiners were below detection limit, while in the latter they were elevated (GUAY & DANDERAND, 1986).

Chromium concentrations are elevated at certain stations (figure 8). The higher concentration at station 15 may have its source from the industrial area near Varennes on the south shore. The higher concentrations in spottail shiners at station 27 (1st date) could be the very local influence of the marina of Ste.Anne de Sorel, where these specimens were caught. These higher concentrations were not found in yellow perch caught just upstream from this marina; therefore, a more upstream situated source seems unlikely. Shortly after the 1st sampling date, the marina closed for the winter, which could be a reason for the lower values in spottail shiner at the 2nd sampling date. The higher concentrations at station 28 could have their source from the industrial area of Sorel. The St.François River (station 43) seems to be a source of chromium and, as will be seen later, of other metals. Levels of cadmium, chromium, copper, nickel and arsenic were elevated in both fish species. AUGER (1980) has reported the presence of high levels of heavy metals in the St.François water column and sediments. He stated as sources mining and industrial activities within the river's basin

Copper concentrations were well above detection limit at all stations (figure 9) and levels of about 0.5 ppm seem to reflect normal body concentrations, which can be due to the fact that copper, in trace amounts, is an essential element for a number of enzymes (FÖRSTNER & WITTMANN, 1979). We have no explanation for the hiogher levels at station 22, but the higher concentrations at station 27 to 29 could again be the influence of the industrial activities at Sorel. The difference between the two sampling dates at station 27 reflects maybe the short turnover time of copper in these fish species or perhaps that the copper is not taken up by the fish but just superficial attached to the mucus on the skin.

Nickel concentrations in fish show the same patterns as chromium concentrations (figure 10): high levels at station 15, 27 (1st date; spottail shiner), 28 and 43. Unlike sediments, where the same patterns could be the result of similar behaviour of these metals in the aquatic environment, the patterns in fish display that these two metals have the same sources such as industrial dyes, metal plating, etc. (FÖRSTNER & WITTMANN, 1979).

Lead concentrations are situated around the detection limit (figure 11). Point sources seem to exist at station 27 (the marina of Ste.Anne de Sorel), station 41 (Richelieu River) and station 43 (St.François River). The difference between the two sampling dates for spottail shiner at station 27 may, like chromium and nickel, again be due to the closing of the marina, especially since most pleasure-crafts use leaded gasoline. ANONYMOUS (1983) cites that the Richelieu River discharges 100 kg of lead per day into the St.Lawrence River, which could explain the high levels at station 41. The high levels at station 1 to 4 could be the influence of the industries near Valleyfield and at the stations 11 to 15 the influence of the MUC, although the station 11, 12 are upstream of the MUC sewage outlet.

Zinc is an essential element as a cofactor for several enzymes and the levels of this metal are much higher in the fish than for any other metal (figure 12). Although the levels differ significantly between the stations, the relative fluctuations, in comparison with the fluctuations of the others metals, seem to be minor and are probably natural in origin. There is, however, a large difference between bodyburden contents for spottail shiner and yellow perch; the latter being about 50% lower.

The background levels in forage fish for arsenic seem to be below detection limit. Although at 19 of the 22 stations, the levels were at or above this detection limit (figure 13), so no real point source for arsenic is evident in the investigated area.

Selenium displays roughly the same patterns as zinc (figure 14). Little variation, although most differences are significant, between the stations amongst the same species. There are large differences between the two species, the bodyburden levels in spottail shiner being about 2 times higher than in yellow perch. In trace amounts, it is essential for growth, fertility and disease prevention (FÖRSTNER & WITTMANN, 1979).

1.3.3 Correlation Of Bodyburden Contents Of Contaminants Between Yellow Perch And Spottail Shiner

In the report of the 1984 study, it was concluded that yellow perch is a good replacement species for spottail shiner, as yellow perch was found at stations where no spottail shiner was caught, and the bodyburden levels in both species caught at the same station showed resemblance (GUAY & DANDERAND, 1986). In this 1986 study, yellow perch has also been used to replace spottail shiner at several stations. The 1986 data have been added to the 1984 data, to evaluate if there is a significant correlation of bodyburden levels for the different contaminants between the two species. Although we are not dealing with a true cause-effect relationship or regression (SOKAL & ROHLF, 1981), we did feel justified (PIERRE LEGENDRE, pers.com.) to apply regression equations to the data set in order to make predictions on contaminant levels from one species to the other. In other words, an regression equation can be used to predict, from existing yellow perch data, what would be the concentrations in spottail shiner, when only yellow perch has been caught at a particular station.

A regression equation is, however, only calculated when the correlation is significant. When the intercept was found not to be significant, the equation without the intercept is also given.

The correlations between the two species for the different contaminants are presented in the figures 15 to 24. Cadmium is not included, since too many values were below detection limit, as is the case for lead, but the latter is included to illustrate the difference

between the two species. Zinc is not included, because there was a large difference in precision within the 1984 data. Arsenic and selenium are not presented, because only 4 data were available from the 1986 study (in 1984, As and Se were not analysed).

1.3.3.1 Organochlorinated Compounds

The correlation for DDE (figure 15) is highly significant. Although the intercept is not significant, omitting it diminishes the correlation which becomes less significant (omitting the intercept will always result in a decrease of the correlation, but it gains a degree of freedom and the result can be more significant).

The correlation for HCB (figure 16) is highly significant, but is driven by the one high concentrations. However, the nonparametric Spearman correlation was found to be also highly significant (R**2 = 0.88). Since the slope is based only on a few data points, it would be a little premature to state that yellow perch is accumulating HCB more than spottail shiner. The figure does show that when concentrations of HCB are low or elevated in yellow perch, they follow the same pattern in spottail shiner, which means that these young-of-the-year fish are good indicators for ambient concentrations of HCB in the water column.

The PCB regression between spottail shiner and yellow perch has a slope very close to one (figure 17), which means that they accumulate PCB's more or less at the same rate. The correlation is all the more significant, because of one high value, but even without this value the correlation remains highly significant (equation with intercept R**2 = 0.61, p <0.01; equation without intercept R**2 = 0.48, p <0.05).

From the 1986 analytical results, it became clear that there is very little variation within a station, especially for the organic contaminants. This is probably caused by the fact that a single analysis is carried out on a batch of many individuals (i.e. yellow perch 12-24; spottail shiner 50-130). Individual variation will be attenuated between replicates, as each replicate (batch) analysed results in a mean value. This fact allowed us to check the results of the organic contaminants of the 1984 study for some outliers. Among those found, a few turned out to be mistakes. The remaining outliers may have been the result of the sampling strategy in 1984: a station was divided into different strata and the fish caught at a station were analysed per stratum (GUAY & DANDERAND, 1986), as opposed to the 1986 strategy, where all fish were mixed for a particular station before splitting into 5 replicates.

In figure 18, the PCB correlation between the two species is again presented, but this time based on means per station without the remaining outliers. For these latter equations, the R**2 is somewhat higher, but the significance level stays about the same compared with

the former equations (figure 17) and the slopes of both are a little steeper. Still, they do differ not very much from 1. Altogether the influence of the outliers on the correlation between spottail shiner and yellow perch seems to be, in this case, negligible. These details are emphasized, because it is very important to check data for outliers and evaluate if they make sense. Also the influence of an outlier on the correlation should be investigated carefully.

1.3.3.2 Heavy Metals

The correlation between spottail shiner and yellow perch for mercury concentrations is presented in figure 19. This figure shows a similar pattern as figure 16 for HCB: many observations with low concentrations of mercury and only a few with higher concentrations. If one only looks at the cloud of points at low concentrations, then it is clear that there is no correlation between the two species for this concentration range. The regression is, therefore, mainly based on the other points, representing the higher concentrations. Again, as in the case with PCB's, the slope of the regression equation is very close to one, which may indicate that yellow perch and spottail shiner are concentrating mercury at the same rate, and that yellow perch can replace spottail shiner when the latter is absent at a certain station.

For chromium, there is a very good correlation between spottail shiner and yellow perch (figure 20). If the points below the detection limit are excluded, then the other ones fall almost on a straight line. However points below detection limit are included, because they give some indicative information on the bodyburden content.

As can be seen from figure 21, there is no significant relationship for copper between spottail shiner and yellow perch, although there is some indication ($p<0.10$) that bodyburden contents in both fishes are correlated.

Nickel seems to be an good example of a metal which is concentrated in both species but not in the same amount (figure 22). Yellow perch concentrates nickel about 1.5 to 2 times more than spottail shiner. In the case of nickel we can give an example of what the influence of a high value is on the correlation coefficient and on the regression equation. In figure 23 is presented the correlation without the one high value from figure 22. The correlation is smaller but still significant. The slopes of both regressions are a little steeper, although for the equation without the intercept, the difference is small (slope=0.63 compared with 0.53) and not significant.

Finally, in figure 24, the correlation between the two species for lead is given. No definite conclusions can be drawn from this figure about a correlation between both species. One conclusion,

however, can be that spottail shiner is concentrating lead more than yellow perch: in spottail shiner, only one value is below the detection limit while for yellow perch only one is above it.

1.3.4 Relation Between Contaminant Concentrations, Length And Lipid In Forage Fish

The mean lengths with the 95% confidence limits are displayed in figure 25 and table 3. It is clear from this figure that there are highly significant differences in length between the stations. This is confirmed by the ANOVA results:

Spottail shiner
Fs = 13.31 DF nominator = 16; DF denominator = 4428
F0.001[15,infinity] = 2.51

Yellow perch
Fs = 4.68 DF nominator = 9; DF denominator = 795
F0.001[9,infinity] = 3.10

For these reasons we analysed length groups versus contaminants levels within a station as listed in table 4. Regression analyses were carried out, and when $p < 0.10$, their equation has been presented in table 5.

Table 3. Descriptive statistics of lengths (mm) of spottail shiner and yellow perch for each station analysed. STDEV = standard deviation; SEM = standard error of the mean; CONF.LIM. = confidence limit.

a. spottail shiner
the stations 28, 29, 31, 32, 42 and 46 were not analysed

STATION CODE	COUNT	MEAN	STDEV	SEM	95% CONF.LIM.	MEAN-95% CONF.LIM.	MEAN+95% CONF.LIM.
01	244	40.6	13.74	0.88	1.72	38.9	42.3
03	167	47.6	10.36	0.82	1.61	46.0	49.2
04	152	48.7	9.82	0.83	1.62	47.1	50.3
11	176	45.8	11.12	0.85	1.67	44.1	47.4
12	187	45.0	11.72	0.87	1.70	43.3	46.7
13	285	39.3	15.47	0.91	1.79	37.6	41.1
14	223	42.2	12.38	0.87	1.71	40.5	43.9
15	175	46.0	10.69	0.82	1.61	44.4	47.6
16	223	43.2	12.71	0.85	1.67	41.5	44.9
21	486	37.1	20.75	0.94	1.84	35.3	39.0
22	101	40.1	7.09	0.76	1.49	38.6	41.6
25	535	37.8	22.02	0.95	1.86	35.9	39.7
27(1st date)	319	44.6	16.82	0.94	1.84	42.8	46.4
27(2nd date)	315	45.5	15.87	0.90	1.76	43.7	47.2
30	240	41.1	14.00	0.90	1.77	39.3	42.8
41	214	47.1	12.10	0.84	1.64	45.5	48.8
43	284	46.0	14.95	0.89	1.74	44.3	47.7

b. yellow perch
the stations 1, 3, 4, 11 to 16, 21, 25 and 41 were not analysed

STATION CODE	COUNT	MEAN	STDEV	SEM	95% CONF.LIM.	MEAN-95% CONF.LIM.	MEAN+95% CONF.LIM.
27(1st date)	48	63.6	7.91	1.55	3.03	60.6	66.6
27(2nd date)	69	67.8	7.41	1.21	2.37	65.4	70.2
28	65	68.2	5.34	1.13	2.21	66.0	70.4
29	88	62.6	6.86	0.94	1.85	60.7	64.4
30	96	62.5	7.22	0.97	1.90	60.6	64.4
31	76	60.8	6.55	1.00	1.97	58.9	62.8
32	80	63.7	7.39	1.05	2.06	61.6	65.7
42	77	65.9	7.80	1.13	2.22	63.7	68.1
43	92	62.2	6.33	0.87	1.71	60.4	63.9
46	82	58.5	7.21	0.98	1.93	56.6	60.4

Table 4. Lipid and contaminant concentrations (ppm, unless stated otherwise) for different size groups of spottail shiner and yellow perch. The detection limit is given below each contaminant. Note: one should not pay attention to the significance of the decimals; for uniformity all columns have the same layout.

	>-----SPOTTAIL SHINER-----<				>----------YELLOW PERCH----------<					
length group (mm)	35-<40	40-<45	45-<50	50-<55	55-<60	60-<65	65-<70	70-<75	75-<80	80-<85
lipid % wet weight	2.610	3.430	5.290	5.620	2.420	2.550	3.250	3.520	2.950	3.860
HCB 1 ppb	0.500	0.500	5.000	6.000	0.500	0.500	0.500	0.500	0.500	1.000
DDE 0.001	0.008	0.013	0.016	0.026	0.006	0.008	0.008	0.007	0.009	0.011
PCB's 0.01	0.130	0.260	0.300	0.330	0.190	0.210	0.240	0.200	0.270	0.300
A1254 0.01	0.110	0.160	0.210	0.240	0.150	0.170	0.190	0.160	0.220	0.240
A1260 0.01	0.020	0.100	0.090	0.090	0.040	0.040	0.050	0.040	0.050	0.060
Heptachlor 1 ppb	0.500	7.000	5.000	4.000	0.500	0.500	0.500	0.500	0.500	0.500
Aldrin 1 ppb	0.500	7.000	5.000	4.000	0.500	0.500	0.500	0.500	0.500	0.500
Hg 0.01	0.050	0.060	0.050	0.040	0.040	0.040	0.040	0.040	0.040	0.050
Cr 0.2	0.210	0.100	0.100	0.100	1.780	1.310	1.920	1.980	2.130	1.640
Ni 0.05	0.130	0.130	0.160	0.160	1.330	1.030	1.460	1.510	1.600	1.240
Zn 0.2	44.200	44.200	44.000	43.000	24.500	22.900	23.400	22.500	22.400	21.900
Cu 0.2	0.480	0.590	0.670	0.660	1.110	1.050	1.020	0.970	1.020	1.020
Cd 0.02	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Pb 0.10	0.050	0.050	0.120	0.110	0.050	0.050	0.050	0.050	0.050	0.050
As 0.05	0.025	0.060	0.070	0.070	0.150	0.160	0.130	0.170	0.160	0.150
Se 0.05	0.410	0.520	0.670	0.690	0.460	0.450	0.360	0.390	0.380	0.390

Table 5. Linear regression equations between length (mm) and lipid (% wet weight) or contaminants (ppm), and between lipid and contaminants. Spottail shiners (nohu) came from station 16 and yellow perch (pefl) from station 28. A1254 = Arochlor 1254, A1260 = Arochlor 1260, n.s. = not significant.

correlation of LENGTH (X) with (Y)	of LIPID (X) with (Y)	SPECIES	REGRESSION EQUATION Y =	SIGN.LEVEL
LIPID		Nohu	2.18e-01X -5.45	<0.05
DDE		Nohu	1.14e-03X -3.50e-02	<0.05
	DDE	Nohu		n.s.
PCB's		Nohu	1.28e-02X -3.15	<0.10
	PCB's	Nohu	5.50e-02X -0.02	<0.10
A1254		Nohu	8.8e-03X -0.212	<0.01
	A1254	Nohu	3.87e-02X -1.6e-02	<0.05
A1260		Nohu		n.s.
Heptachlor		Nohu		n.s.
Aldrin		Nohu		n.s.
Hg		Nohu	-1.4e-03X +0.115	<0.10
Cu		Nohu	1.24e-02X +4.82e-03	<0.10
Ni		Nohu	2.4e-03X +0.038	<0.10
Zn		Nohu		n.s.
As		Nohu		n.s.
Se		Nohu	1.98e-02X +0.309	<0.05
LIPID		Pefl	4.95e-02X -0.352	<0.05
DDE		Pefl	1.54e-04X -2.55e-03	<0.05
	DDE	Pefl		n.s.
PCB's		Pefl	3.94e-03X -3.90e-02	<0.05
	PCB's	Pefl		n.s.
A1254		Pefl	3.26e-03X -3.8e-02	<0.05
A1260		Pefl	6.86e-04X -9.9e-04	<0.10
Hg		Pefl		n.s.
Cr		Pefl		n.s.
Cu		Pefl		n.s.
Ni		Pefl		n.s.
Zn		Pefl	-9.09e-02X +29.23	<0.05
	Zn	Pefl	-1.24X +26.8	<0.10
As		Pefl		n.s.
Se		Pefl		n.s.

From table 5, it is clear that length and lipid within a station are correlated. This relationship is, however, only true for within stations. When the means of the lengths at different stations are compared with the means of the lipids very little correlation is found

(table 5 and 6). This correlation between length and lipid within a station makes it difficult to separate them with regards to their relationship with the contaminant concentrations. Length, through its relationship with age, is a measure of the exposure time, while lipid concentration influences the uptake of lipophilic contaminants, such as DDE, HCB, PCB's and methylmercury. For the other metals, there is no ground to expect a relationship with lipid contents. In all cases, however, the correlation between length and a contaminant was found to be higher than between lipid and a contaminant (this cannot be deduced as such from the significance levels in table 5). So exposure time may well be more important than lipid contents.

The equations can roughly be interpreted as follows: the slope is an indication of the change in contaminant concentration (ppm) per length increase (mm); for example the levels of PCB's in spottail shiner will increase 0.128 ppm with a length increase of 10 mm.

Because we suppose that the forage fish are a good indicator for ambient concentrations of contaminants in the water column, the slope of the regression of a contaminant on the length will be steeper or flatter as the concentrations in the water are higher or lower. Therefore, a regression equation for one station can, in principle, not be applied to an other station.

It is not our purpose at this stage to correct the data for length, since we have carried out the regression analyses only to provide some qualitative details. It could ,however, be an important factor when one compares stations. The maximum difference between mean lengths that we have found is 11.6 mm for spottail shiner and 9.7 mm for yellow perch, although SUNS et al. (1985) have analysed young-of-the-year spottail shiners over the years with length differences of up to 35 mm.

It is difficult to propose here a sampling strategy. We took random samples from the collected fish and found length differences between stations. One could also select spottail shiner and yellow perch within a defined size group and, by doing so, select a part of the yearclass, which may be biased. The disadvantages of this approach could, however, be a loss of certain stations, because there might be no fish, or hardly any, in the desired size class. SUNS (pers.com.) is now selecting young-of-the-year spottail shiners of over 50 mm. This size class would certainly give problems for the St.Lawrence River, since these are rare there. Besides the loss of stations, one does not know what is the cause of the length differences between the different stations. The growth during the sampling period does not seem to be all that important in the case of spottail shiner (compare the results of the two dates for station 27 in table 3), and explains only a part of the variation for yellow perch. These differences in length could be due to differences in spawning time and/or environmental factors. We do not want, or cannot, make predictions if individuals of the same length at different stations have had the same efficiency in contaminant uptake. Up until now we can only assume this. Maybe, because we find

significant correlations between two species, the influence of length within a species is negligible.

1.3.5 Correlation Between The Different Parameters In Forage Fish

The correlation coefficients of comparison between the various parameters (length, lipid, organics and heavy metals) for both species are presented in table 6 and 7 (1986 data only). Aldrin, Heptachlor and cadmium are not included, since too many values were below the detection limit. For the same reason, HCB and lead are also excluded, but only for yellow perch.

There are some differences between the two species. For example, there is a significant correlation between the lipid and organochlorine residues in spottail shiner, which was not found in yellow perch. However, instead of discussing the differences between the two species, we shall explore more their similarities, because if two species display the same correlation between two parameters, then it is more likely that a true relationship between the two parameters exists.

For both species, there is a significant correlation between DDE and PCB's. It seems unlikely that these two contaminants have the same sources, and it is probably more a question of the omnipresence of both and their similar lipophilic caracteristics.

More can be said about the relationship between chromium and nickel. This is an example of two metals, which have very often the same sources (e.g. fertilizers, steel work foundries, plating, finishing) although chromium is more used than nickel (FÖRSTNER & WITTMANN, 1979). The correlation between chromium and nickel is presented in the figures 26 and 27. From these figures it is clear that the levels of chromium are higher than those of nickel in both species, and that the bodyburden contents are highly correlated.

The correlation between PCB's and selenium was also found to be significant in both species (figure 28 and 29). We found no information in the literature on the possible cause of this correlation. It seems unlikely that PCB's and selenium have the same source, or behave similarly in the environment. Although selenium is very toxic, in trace amounts it has a known detoxification function through its antagonistic effect on other metals, such as cadmium (VAN PUYMBROEK et al., 1982) and copper (WINNER, 1984). Selenium binds these metals to form insoluble selenites and its protecting effect appears to be general in character. In nature, selenium concentrations were found to be highly correlated with methylmercury concentrations in sea mammals (KOEMAN et al., 1975). It seems that selenium uptake can be regulated to a certain extent. Because of the correlation that is found between PCB's and selenium, it may be that selenium has a antagonism with PCB's. If the latter is true then low concentrations of PCB's might already have a harmful effect on the

fish, since it has to activate the uptake of selenium to detoxify PCB's.

Table 6. Pearson's coefficients of correlation between the different parameters for spottail shiner.
Critical values R0.05[1,15] = 0.482 R0.01[1,15] = 0.606.
Source: STEEL & TORRIE (1980). * = p<0.05; ** = p<0.01

	lipid	length	HCB	DDE	PCB	A1254	A1260	Hg	Cr	Cu	Ni	Pb	Zn	As	Se
lipid	1.00														
length	0.22	1.00													
HCB	-0.06	0.47	1.00												
DDE	0.84 **	0.16	-0.02	1.00											
PCB	0.59 *	0.02	-0.01	0.85 **	1.00										
Arochlor 1254	0.55 *	0.03	0.01	0.81 **	0.99 **	1.00									
Arochlor 1260	0.67 **	0.01	-0.07	0.90 **	0.96 **	0.92 **	1.00								
Hg	-0.24	0.31	0.88 **	-0.24	-0.16	-0.10	-0.20	1.00							
Cr	-0.22	0.31	-0.13	-0.27	-0.44	-0.49 *	-0.21	-0.18	1.00						
Cu	-0.19	0.02	-0.18	-0.15	-0.06	-0.10	0.03	-0.17	0.63 **	1.00					
Ni	-0.13	0.12	-0.21	-0.18	-0.25	-0.30	-0.12	-0.36	0.88 **	0.67 **	1.00				
Pb	-0.06	0.38	-0.06	0.02	-0.15	-0.15	-0.14	-0.14	0.61 **	0.38	0.46	1.			
Zn	0.37	-0.07	-0.03	0.54 *	0.65 **	0.66 **	0.58 **	-0.01	-0.26	-0.18	-0.21	0.06	1.00		
As	-0.52 *	0.01	-0.15	-0.43	-0.10	-0.09	-0.09	0.07	0.19	0.32	0.22	-0.07	-0.09	1.00	
Se	0.32	-0.03	0.39	0.39	0.64 **	0.64 **	0.59 *	0.34	-0.38	-0.06	-0.20	-0.39	0.46	-0.04	1.00

Table 7. Pearson's coefficients of correlation between the different parameters for yellow perch.
Critical values R0.05[1,8] = 0.632 R0.01[1,8] = 0.765.
Source: STEEL & TORRIE (1980). * = p<0.05; ** = p<0.01

	lipid	length	DDE	PCB	A1254	A1260	Hg	Cr	Cu	Ni	Zn	As	Se
lipid	1.00												
length	-0.19	1.00											
DDE	0.07	0.58	1.00										
PCB	0.35	0.51	0.72 *	1.00									
Arochlor 1254	0.33	0.56	0.73 *	1.00 **	1.00								
Arochlor 1260	0.42	0.29	0.61	0.94 **	0.91 **	1.00							
Hg	-0.10	0.30	0.27	-0.08	-0.05	-0.15	1.00						
Cr	-0.10	0.00	-0.55	-0.25	-0.27	-0.19	0.05	1.00					
Cu	-0.19	0.07	-0.16	0.18	0.20	0.09	-0.50	0.15	1.00				
Ni	0.03	0.04	-0.52	-0.14	-0.15	-0.09	0.08	0.68 **	0.18	1.00			
Zn	-0.49	0.21	-0.42	-0.17	-0.12	-0.31	-0.14	0.35	0.78 *	0.37	1.00		
As	-0.09	0.29	-0.26	0.28	0.28	0.23	-0.40	0.64 *	0.72 *	0.68 *	0.62	1.00	
Se	-0.04	0.64 *	0.57	0.82 **	0.85 **	0.66 *	-0.24	-0.24	0.41	-0.17	0.27	0.41	1.00

1.3.6 Comparison Of Contaminants In Forage Fish Between 1984 And 1986

The comparison of some contaminants between 1984 and 1986 are presented in figure 30 to 34. The comparable station codings are given in table 8.

Table 8. Station codes in 1984 and 1986.

1984:	9	11	12	22	23	24	25	26	27
1986:	1	3	4	11	13	25	22	31	32

Remark: the 1986 stations 25 and 22 were not exactly on the same place as the 1984 stations 24 and 25.

We do not want to go very deep into the comparison between the two years. One has to be careful to make predictions based on only two years of data. SUNS et al. (1985) found after the decrease of organochlorine residues in spottail shiners in the late seventies no diminuation in the eighties. They found, however, moderate fluctuations from year to year. So we shall only describe here what is visualized in the figures, without any extrapolations about future trends. Besides the waterlevel of the St.Lawrence River, which was lower in 1984 (GUAY, pers.com.), may be of influence for the uptake from year to year, since the discharge could be an important dilution factor.

The 1984 and 1986 data for p,p'-DDE are presented in figure 30. At all stations in 1986 the values were much lower at most stations, and at some the same as in 1984. This may be a real trend, because DDT is nowadays seldom used in Canada.

HCB concentrations are less in 1986 compared with 1984 (figure 31). The lower values are especially evident at station 11 and 12, where we have a point source in the St.Louis River.

PCB's are considerably lower in 1986, at station 11 near Beauharnois, where the highest levels were found in 1984 (figure 32). For the other stations, some of them are higher, while others are lower. In general, however, and allowing for some fluctuations the values of 1984 and 1986 are in agreement, indicating that temporal trends can be evaluated in the future.

Mercury levels have hardly changed from 1984 to 1986 (figure 33). These results are in accordance with the temporal patterns found in the Great Lakes, where no any decrease has been found after the decline in the seventies (SUNS et al., 1985).

Finally, figure 34 displays the comparison for lead between 1984 and 1986. It seems that a possible temporal analysis for lead will give difficulties, as stations were not comparable between the two years: stations, which were high in 1984, were low in 1986, and vice versa. Only station 22 gives the same bodycontents in both years.

1.4 DISCUSSION

In this discussion we highlight 3 points of interest: first, the use of forage fish as a bioindicator of contaminants in the aquatic ecosystem; second, the spatial patterns of contaminants in the investigated area, and third, the temporal trends of bodyburden contents of contaminants.

From the results presented in this report, it is clear that spottail shiner and yellow perch concentrate most contaminants well above detection limit, and to much higher levels than in the ambient water column. It seems a good tool to investigate spatial

contamination patterns, as within station variation is also minimal. Unfortunately, young-of-the-year fish are little used as a bioindicator, making comparisons with results from other contamination studies difficult. Moreover, most contaminant measurements on fish were done using muscle tissue (FÜRSTNER & WITTMANN, 1979). In the Great Lakes, forage fish are extensively used as a bioindicator (SUNS et al.,1983,1985; SUNS & REES, 1978), while some limited use of this biomonitoring tool is made in Saskatchewan (MUNRO, 1985).

We would like to emphasize some major advantages of using forage fish over adult fish and its complementary value next to sediment and water analysis. When using forage fish, one has no problems associated with, for instance, age determination, maturity and migration. The former two influence the homogeneity of a sample, and the latter one integrates contamination over a much larger area, resulting in a relative lack of site-specificity. Furthermore, a sample of forage fish consists of many individuals, minimizing the influence of individual variation on the final results. Consequently, forage fish give a very good estimate of the contamination residues in a population under study, hence its value as a biomonitoring tool.

Compared with sediments, forage fish reflect the amount of contaminants in the watercolumn at a specific place, while sediments integrate contaminants from an relatively unknown area over a relatively unknown period of time, without providing any indication on the bioavailability of contaminants, as they are bound to and buried in the sediments.

When compared with water analyses, forage fish intergrate contaminants over a, by approximation, known period of time, while a water sample represents only a snapshot, which has to be taken much more often to allow for seasonal fluctuations. Moreover, forage fish concentrate most contaminants up to a level well above the detection limit, while in watersamples contaminants such as PCB's and p,p'-DDE are often below detection limit (GERMAIN & JANSON, 1984).

The main objective of our study was the investigation of spatial patterns of contaminants in Lake St.Pierre. In general, it can be stated that concentrations of most contaminants in the forage fish change little, as one moves from up- to downstream of Lake St.Pierre. The PCB levels, however, drop considerably when stations 31 and 32 at the outlet are compared with stations 25 to 30 at the upstream end of the lake.

The only north shore tributary analysed, the Maskinonge River, does not seem to have any important source of the contaminants under investigation. All levels were below those found at all other stations. The contaminant composition of the tributaries on the south shore also differ from one to another. The levels of organic contaminants and lead are the highest in the Richelieu River, while the Yamaska River has higher levels of mercury. GOULET & LALIBERTÉ (1982a, 1983) also found that levels of DDE, HCB and PCB's in white sucker (Catostomus commersoni) and walleye (Stizostedion vitreum) were

higher in the Richelieu River than in the Yamaska and the St.François River close to where they enter Lake St.Pierre. The St.François River does not transport much organochlorinated contaminants, the levels in the forage fish in this river were among the lowest found in this study and are comparable with the levels found in the Ottawa River (GUAY & DANDERAND, 1986). It does seem to be an source of heavy metals: cadmium was well above detection limit, which was the case at only 3 other stations, and the levels of chromium, nickel, and lead were among the highest found in both this study and the 1984 pilot study (GUAY & DANDERAND, 1986). GOULET & LALIBERTÉ (1982b) did not find elevated levels of heavy metals in the St.François River, compared with the Yamaska and Richelieu River.

To investigate the influence of the MUC outfall, 2 stations (11 and 12) were sampled upstream, and 2 stations (13 and 14) downstream of the outfall. There was no increase in levels from above to below the outfall, as can be seen from figures 3 to 14. On the contrary, the forage fish at station 13, which is immediate below the outfall, have lower bodyburden contents of PCB's, chromium, copper and nickel, than station 12, and levels comparable with station 11. There were observed, however, significant differences in environmental factors between the stations: the stations 11, 12 and 14 had clear water, while at station 13, there was a high concentration of organic matter, which could have influenced the bioavailability of the toxic substances. Especially for the lower levels of PCB's at station 13 compared with the 3 other stations, a lower bioavailability could be a plausible explanation. Another explanation might be that there is a source more upstream of all these stations, which overshadows a possible increase downstream of the MUC outfall.

In the future, when more years of sampling of forage fish will be added to the already existing years of study, an evaluation of temporal trends will be possible. We have compared 1986 with 1984 but explained that one should not try to extrapolate a trend from these results. As stated before from the work on young-of-the-year spottail shiner in the Great Lakes, it is clear that there are some moderate fluctuations in the eighties in the levels of PCB's and mercury after the decline in the seventies (SUNS et al., 1985). There is no explanation for these fluctuations. As possible causes are suggested the occurence of fluctuations in biovailability from one year to the other, because of variations in turbidity and the presence of organic matter, or that rainfall differences changes the washing out of the contaminated soils (K.SUNS pers.com.). Temporal trend analysis may probably only be possible for organochlorine contaminants and mercury. The example with lead, where some stations were found to be below detection limit in 1986 and not in 1984, and vice versa, displays that there could possibly be very little consistency in bodyburden contents of heavy metals at a station over the years.

1.5 CONCLUSIONS AND RECOMMENDATIONS

The analysis of forage fish has proven to be a valuable tool beside sediment and water analysis, since it supplies information on the bioavailability and bioconcentration of toxic chemicals in the St.Lawrence River. However, no conclusions can be drawn on the possible effects of the toxic chemicals on the young fish. Incidently, the purpose of this study was not to make a toxicological investigation, but to evaluate the spatial patterns and, on a longterm basis, the temporal trends of the contaminants in the St.Lawrence River. From the toxicological point of view, the correlation found between the bodyburden contents of PCB's and selenium is worth further investigation.

The use of forage fish might be limited to the evaluation of persistent contaminant residues, and may have little value to investigate the present more degradable pesticides. It has, however, proven to be an useful indicator of some heavy metal contaminants.

Yellow perch seems a good replacement species, when spottail shiner is absent at a certain sampling site. The more lipophilic contaminants are bioconcentrated in the same quantity by the two species, and bodyburden levels seem to be directly comparable. For the heavy metals, a conversion factor is necessary.

Differences in length and lipid content make comparisons between stations more difficult. It is therefore necessary to make accurate length measurements of the individuals, and that they are analysed for lipid content. For spottail shiner, about 50 specimens per sample will be sufficient for length measurements, while for yellow perch the whole sample will have to be measured, as it consists of only 12 to 24 specimens.

Fish should be selected randomly from the catch at a sampling site, because, if only the fish in a certain size class are selected, one does not know in which way the results are influenced. Although these differences have an unknown correlation with the contaminants levels, it can be concluded that using one yearclass and only two species represents a high level of standardization in a biomonitoring program.

The conclusions in this report are qualitative. In a future study it could be of interest to repeat the analyses of size classes in addition to the analyses of random samples. More than one sample per size class should be used to investigate the variation within size classes. One should also try to make an estimation of the relative bioavailability of the contaminants during the exposure time of the forage fish.

In addition to the use of forage fish as a biomonitoring tool, one should try to investigate the impact of contaminants residues on the forage fish and perhaps add experiments in the laboratory. These experiments could consist of studying clearing rates after transfering

fish into clean water. As was stated before, it could be that, for example, some heavy metals are only superficially incorporated in the mucus of the fish. Yellow perch may be more appropriate for these experiments, because it seems less vulnerable to the effects of manipulation than spottail shiner.

Regarding the choice of the sampling sites in the future, one should try to develop a standard monitoring program for the whole St.Lawrence River (Cornwall - Portneuf) to evaluate temporal trends. These stations should be sampled every year, or as a minimum every other year. Detailed case studies on various stretches of the river would evaluate spatial patterns in more detail.

Because spottail shiner is not present all over Canada, comparison with other species, like emerald shiner and golden shiner, is of interest so that a national biomonitoring program can be developed. Ecologically equivalent species allow comparing stations over a wide area, where different species have to be used. In this context, it is important that results become available on bodyburden contents in different species from highly contaminated regions, such as the Niagara River, since interpolating is a more reliable estimation than extrapolating. It is important to know if the regression equations found in this study have predicting value for more contaminated regions.

1.6 REFERENCES

ANONYMOUS, 1970. Chemical Method no.30. Fish Inspection Branch. Department of Fisheries and Oceans, Canada.

ANONYMOUS, 1978a. Final report of the St.Lawrence River Study Committee. Environment Canada and Service de la Protection de l'Envirronnement du Québec. Éditeur officiel du Québec. 209 p.

ANONYMOUS, 1978b. Great Lakes Water Quality Agreement of 1978. International Joint Commission Canada and the United States. 52 p.

ANONYMOUS, 1980. Les méthodes d'analyse des pesticides organochlorés et des biphényl polychlorés dans l'eau, les sédiments, les boues, les sols, les milieux biologiques, l'air et les hydrocarbures. Comité de normalisation des méthodes d'analyse. Bureau d'étude sur les substances toxiques. Ministère de l'Environnement du Québec. pp. 81-104.

ANONYMOUS, 1983. Inventaire des connaissances sur les sources de pollution dans le fleuve Saint-Laurent. Environment Canada. Environmental Protection Service. Internal report. 68 p.

ANONYMOUS, 1984. RS/1 user's guide. Book 2: graphics and statistics. BBN Software Products Corporation. 229 p.

ANONYMOUS, 1985a. Analyse de pesticides organochlorés et biphényles polychlorés dans les jeunes poissons (PS-E-181). Analytical report of Novalab ltd. 44p. Unpublished manuscript.

ANONYMOUS, 1985b. RS/1 for Vax and Microvax. Release 2 features. BBN Software Products Corporation.

ANONYMOUS, 1986. Directory of methodologies. Analytical Methods Division. National Water Research Institute Canada, Canada Centre for Inland Waters. Burlington (Ont.). pp. 16-28.

AUGER, P. 1980. Synthese des études de la qualité de milieu aquatique de la rivière Saint-François. Service de la qualité des eaux, Ministère de l'Environnement du Québec. 69 p.

CHAMPOUX,L. & H.H.SLOTERDIJK. Étude des tendances dans la contamination par le mercure et les biphényles polychlorés des poissons des lac Saint-Louis et Saint-François de 1971 à 1980. Environment Canada, Inland Water Directorate, Water Quality Branch, Quebec Region. In press.

FÖRSTNER, U. & G.T.W.WITTMANN, 1979. Metal pollution in the aquatic environment. Springer-Verlag. 486 p.

GERMAIN, A. & M.JANSON, 1984. Qualité des eaux du fleuve Saint-Laurent de Cornwall à Québec (1977-1981). Environment Canada, Inland Water Directorate, Water Quality Surveys, Quebec Region. 232 p.

GOULET, M. & D.LALIBERTÉ, 1982a. BPC: contamination du milieux aquatique au Québec méridional. Service de la qualité des eaux, Ministère de l'Environnement du Québec. Publication Q.E.-53. Envirodoq ND-83-0018. 57 p.

GOULET, M. & D.LALIBERTÉ, 1982b. Metaux: contamination du milieux aquatique au Québec méridional. Service de la qualité des eaux, Ministère de l'Environnement du Québec. Publication Q.E.-51. Envirodoq ND-83-0017. 115 p.

GOULET, M. & D.LALIBERTÉ, 1983. Pesticides organochlorés: contamination du milieux aquatique au Québec méridional. Service de la qualité des eaux, Ministère de l'Environnement du Québec. Publication Q.E.-54. 82 p.

GUAY, G., 1979. Echantillonage de poissons pour l'étude sur les substances toxiques. Lac Saint-Louis - lac Saint-François. Report submitted to Environment Canada and Fisheries and Oceans, Captain Bernier Laboratory, Longueuil (Que). 21 p.

GUAY, G., 1986. Echantillonage des jeunes poissons de l'année pour l'étude des substances toxiques dans le fleuve Saint-Laurent (tronçon fluvial: Mélocheville - Trois-Rivières). Report presented to Environment Canada, Quebec region, Conservation and Protection.

GUAY, G. & J.DANDURAND, 1986. Utilisation des jeunes poissons de l'année comme bioindicateur des substances toxiques dans le fleuve Saint-Laurent (tronçon fluvial: Cornwall - Portneuf). Final report presented to the Department of Supply and Services and Environment Canada, Inland Waters Directorate. 132 p.

JANSON, M. & H.H.SLOTERDIJK, 1982 Surface water quality data. St.Lawrence River, Quebec 1950 - 1980. Environment Canada, Inland Water Directorate, Water Quality Surveys, Quebec Region. 151 p.

JARRY, V., 1986. Répartition spatiale et effet phytotoxique des contaminants dans les sédiments du lac Saint-Louis (fleuve Saint-Laurent). Memoire de Maitrise es sciences biologiques. Université de Montréal.

KOEMAN, J.H., W.H.M.PEETERS, C.H.M.KOUDSTAAL-HOL, P.S.TJIDE & J.J.M.DE GOEIJ, 1975. Mercury-selenium correlations in marine mammals. Nature (London) 245: 385-386.

LÉVESQUE, F. & C.POMERLEAU, 1986. Contamination de la chair de certaines espèces de la faune aquatique et amphibienne du lac Saint-Pierre par les biphényles polychlorés, le mirex et le mercure (1983 et 1984). Ministère du Loisir, de la Chasse et de la Pêche du Québec, Direction de la faune aquatique, Service des Espèces d'eau fraîche, Québec. Rapp. tech. 86-01. 105 p.

McCANN, J.A., 1959. Life histories studies of the spottail shiner in Clear Lake, Iowa, with particular reference to some sampling problems. Trans. Am. Fish. Soc. 88: 336-343.

MUNRO, D.J., 1985. Report on mercury in Cookson Reservoir. Environment Canada, Inland Water Directorate, Western and Northern Region. 25 p.

NRIAGU, J.O. & M.S.SIMMONS, 1983. Toxic contaminants in the Great Lakes. Advances in environmental science and technology. vol 14. John Wiley & Sons.

PUYMBROECK, S.L.C.van, W.J.J.STIPS & O.L.J.VANDERBORGHT, 1982. The antagonism between selenium and cadmium in a freshwater mollusc. Arch. Environm. Contam. Toxicol. 11: 103-106.

SCOTT, W.B. & E.J.CROSSMAN, 1973. Freshwater fishes of Canada. Bulletin 184. Fisheries Research Board of Canada.

SLOTERDIJK, H.H., 1977. Accumulation des métaux lourds et des composés organochlorés dans la chair des poissons du fleuve Saint-laurent. Rapport technique no. 7. Rapport soumis au comité d'étude sur le fleuve Saint-Laurent par le Ministère du Tourisme, de la Chasse et de la Pêche. Editeur officiel du Québec. 181 p.

SLOTERDIJK, H.H., 1979. La contamination mercurielle chez les poissons des lacs Saint-Louis and Saint-François avec une evaluation des tendences des concentrations de 1971 a 1978. Presente a la conference 'les contaminants dans l'environnement', 14, 15 mai 1979, chateau Frontenac, Québec.

SLOTERDIJK, H.H., 1984. Substances toxiques dans les sédiments du lac Saint-François (fleuve Saint-Laurent, Québec). Environment Canada, Inaland Water Directorate, Quebec Region.

SLOTERDIJK, H.H., 1987. Use of young-of-the-year fish as bioindicator of toxic chemicals to compliment the water quality network in the St.Lawrence River, Quebec Province. In press.

SOKAL, R.R. & F.J.ROHLF, 1981. Biometry. Second edition. W.H.Freeman and company. pp 562-565.

STEEL, R.G.D. & J.H.TORRIE, 1980. Principles and procedures of statistics. Second edition. McGraw-Hill Book Company. p 597.

SUNS, K., G.R.CRAIG, G.E.CRAWFORD, G.A.REES, H.TOSINE & J.OSBORNE, 1983. Organochlorine contaminant residues in spottail shiners (Notropis hudsonius) from the Niagara River. J. Great Lakes Res. 9: 335-340.

SUNS, K., G.E.CRAWFORD & D.D.RUSSELL, 1985. Organochlorine and mercury residues in young-of-the-year spottail shiners from the Detroit River, Lake St.Clair and Lake Erie. J. Great Lakes Res. 11: 347-352.

SUNS, K., G.E.CRAWFORD, D.D.RUSSELL & R.E.CLEMENT, 1985. Temporal trends and spatial distribution of organoclorine and mercury residues in Great Lakes spottail shiners (1975-1983). Ontario Ministry of the Environment. Water Research Branch. Aquatic Contaminants Section. 43 p.

SUNS, K. & G.A.REES, 1978. Organochlorine contaminant residues in young-of-the-year spottail shiners from Lakes Ontario, Erie and St.Clair. J. Great Lakes Res. 4: 230-233

WINNER, R.W., 1984. Selenium effects on antennal integrity and chronic copper toxicity in Daphnia pulex (deGeer). Bull. Environ. Contam. Toxicol. 33: 605-611.

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Figure 33. Comparison of the MERCURY body contents (mg/kg wet weight) between 1984 and 1986. The 1984 station code is used. Bars represent the mean ± standard error of the mean. At stations 9, 11, 12, 22, 23 and 24 the body contents are in spottail shiner where at station 26 the body contents are in yellow perch.

Figure 34. Comparison of the LEAD body contents (mg/kg wet weight) between 1984 and 1986. The 1984 station code is used. Bars represent the mean ± standard error of the mean. At stations 9, 11, 12, 22, 23 and 24 the body contents are in spottail shiner where at station 26 the body contents are in yellow perch.

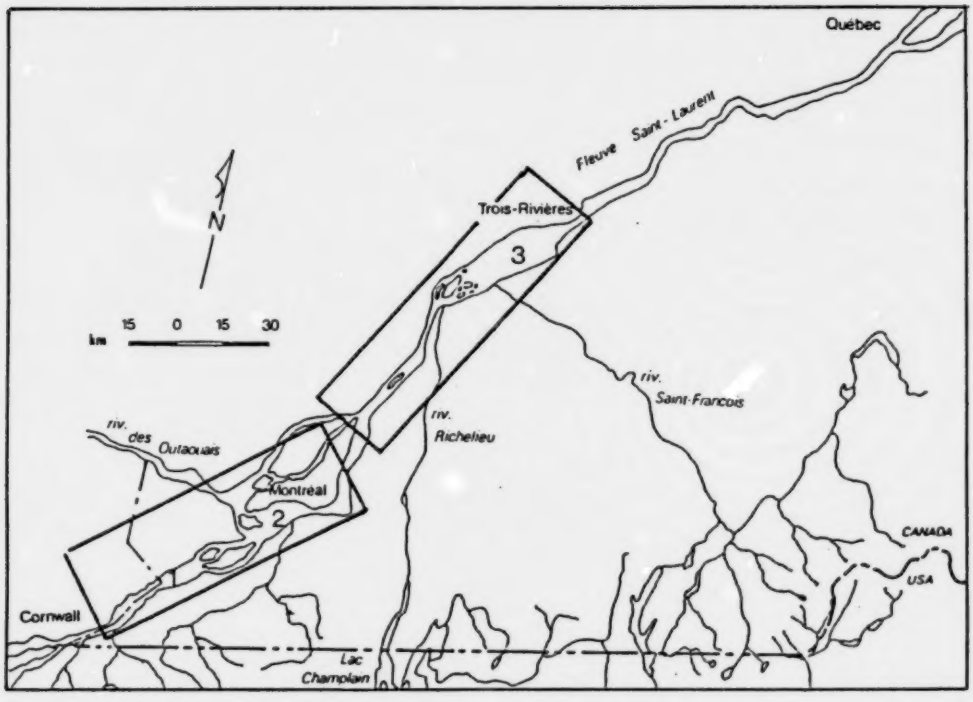


FIGURE 1. The St.Lawrence River from Cornwall to Quebec.
1. Lake St.Francis (lac Saint-François)
2. Lake St.Louis 3. Lake St.Pierre

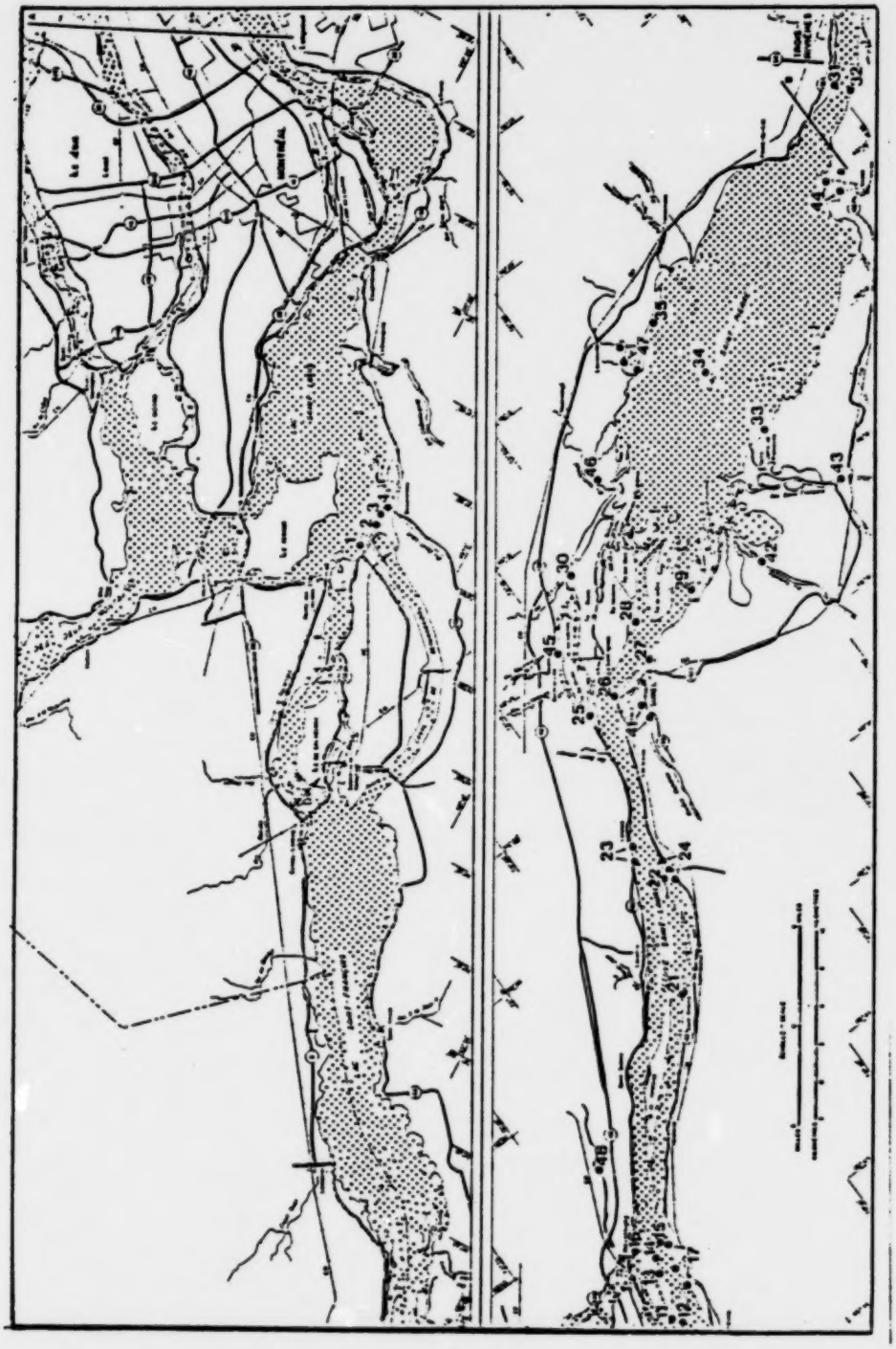


FIGURE 2. Location of the sampling stations

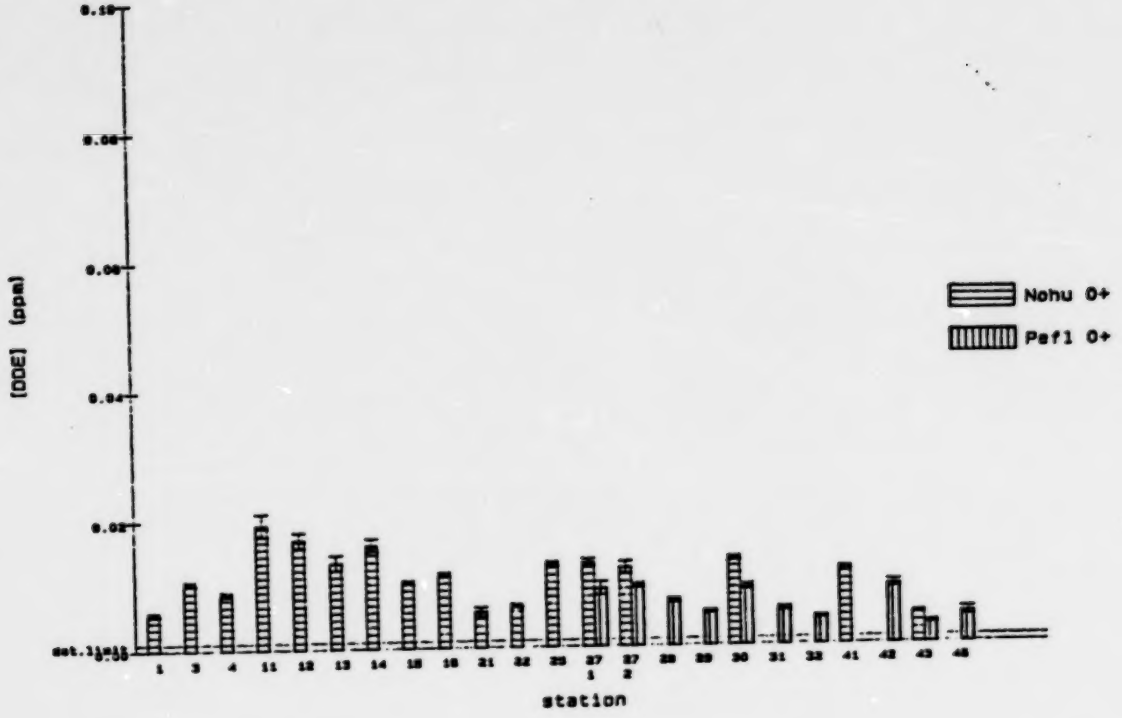


FIGURE 3.

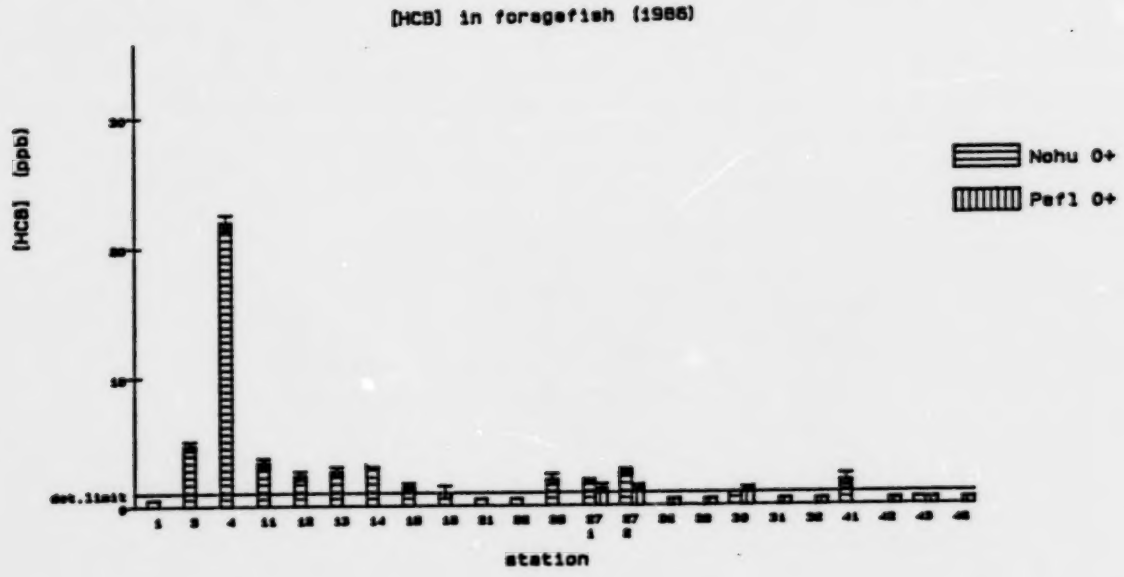
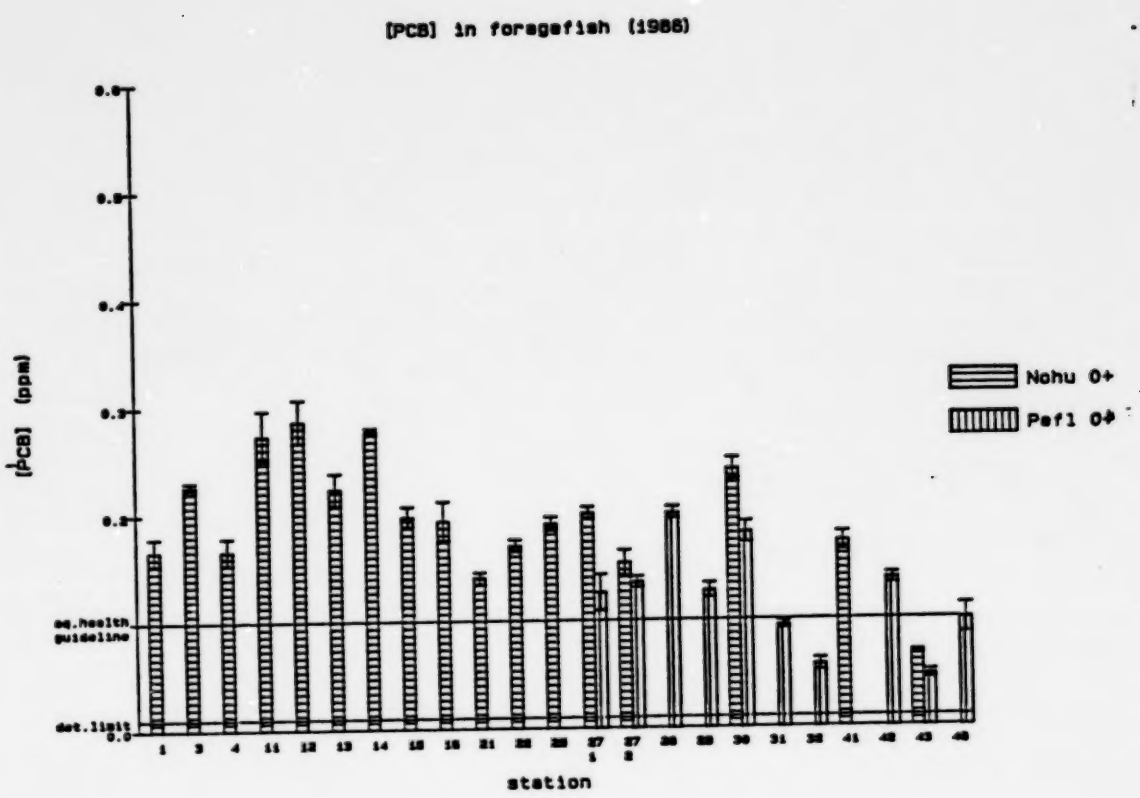


FIGURE 4



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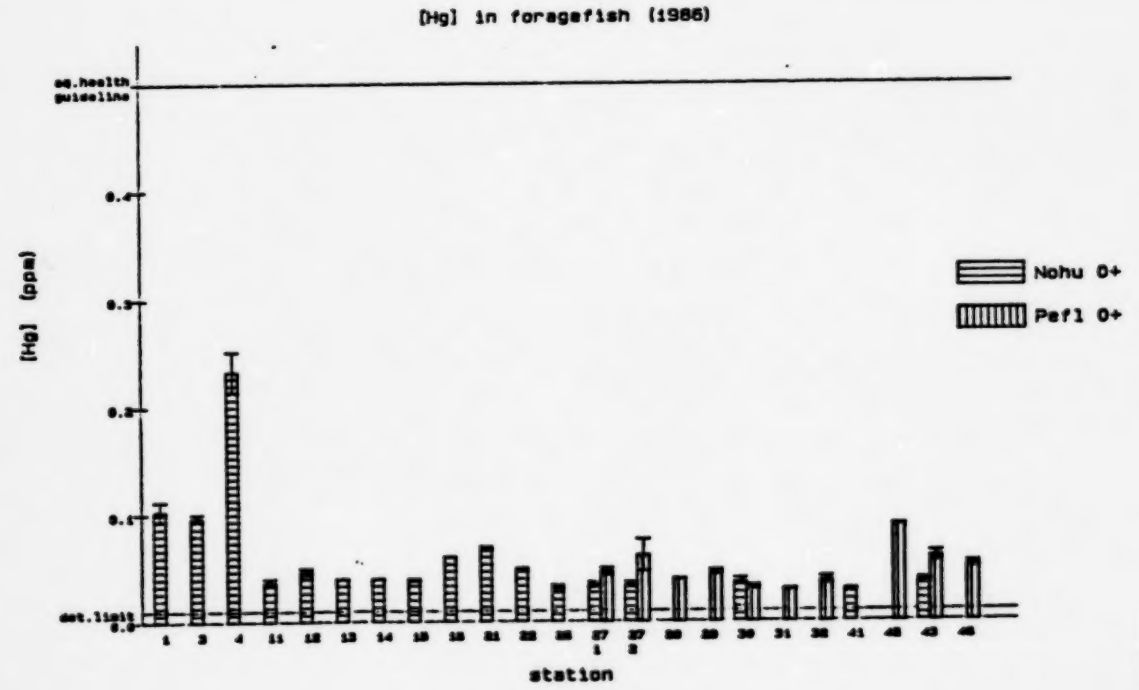

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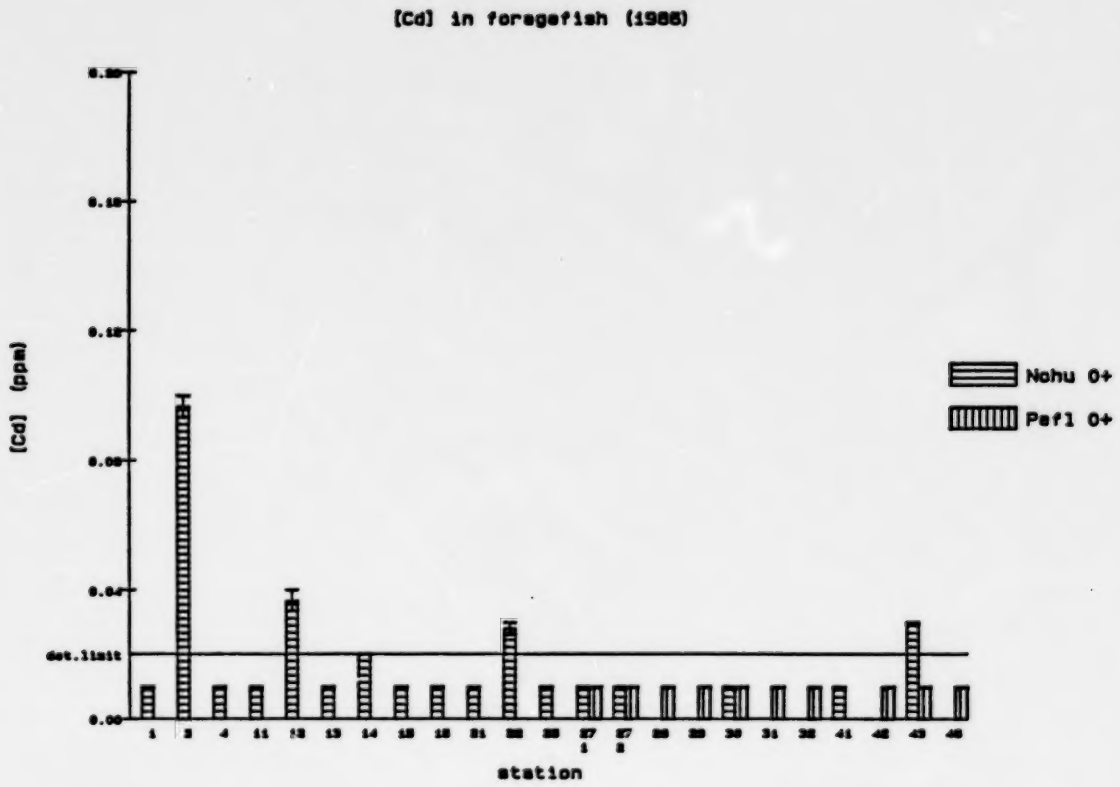

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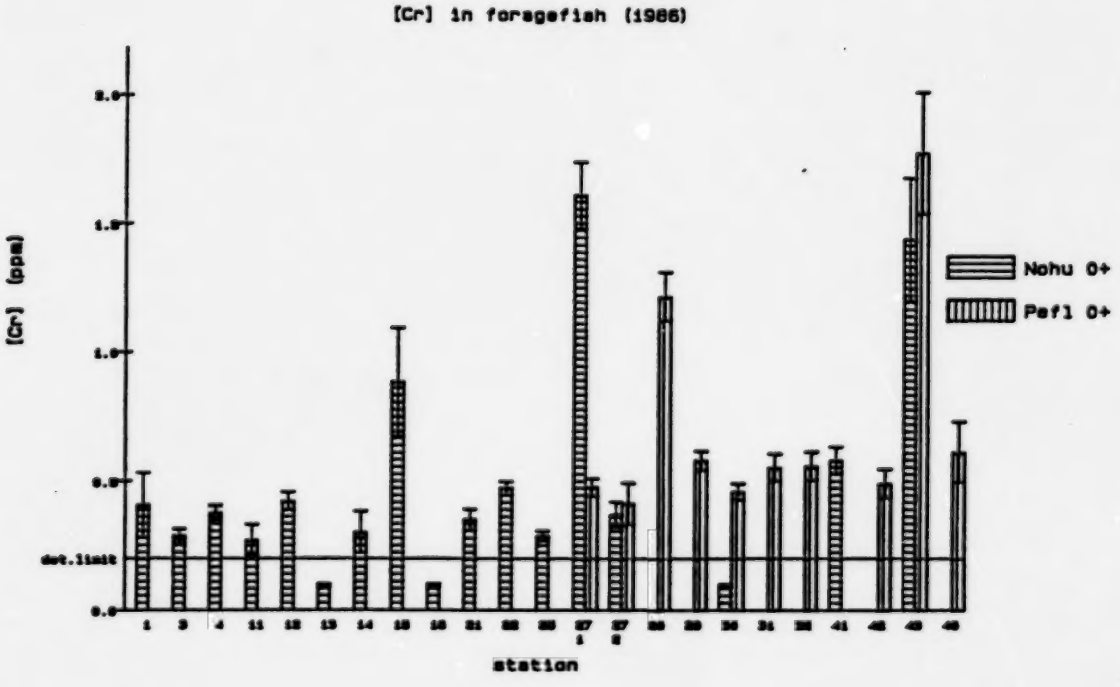

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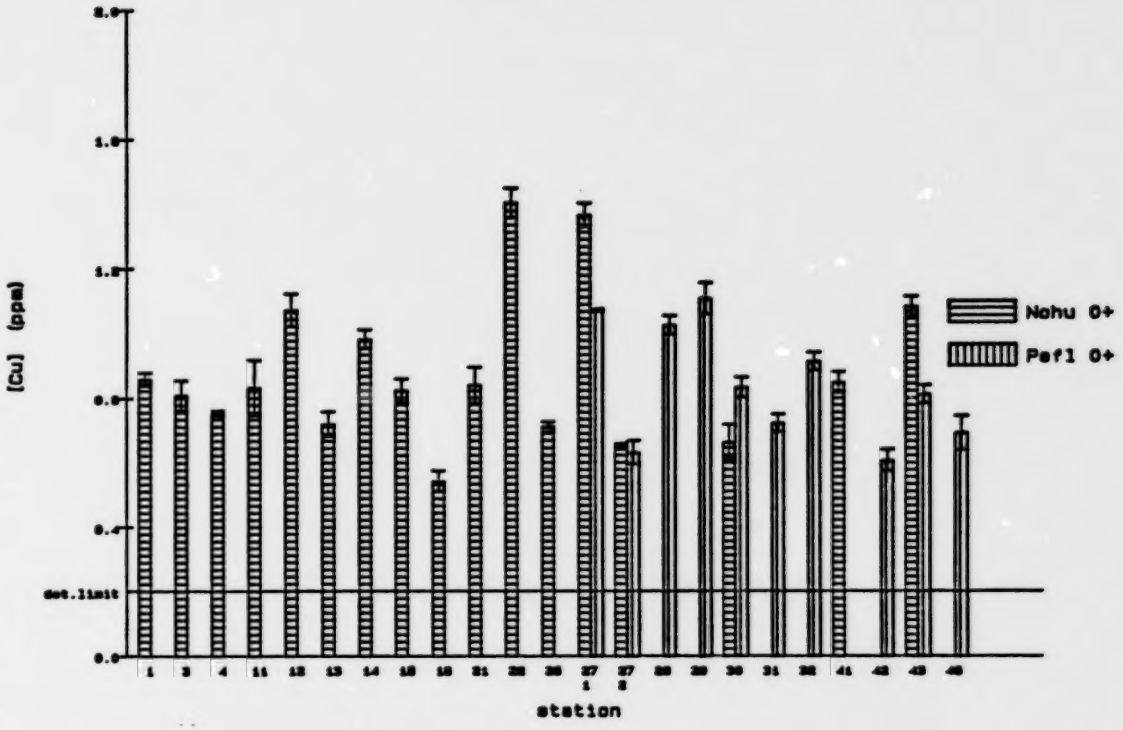

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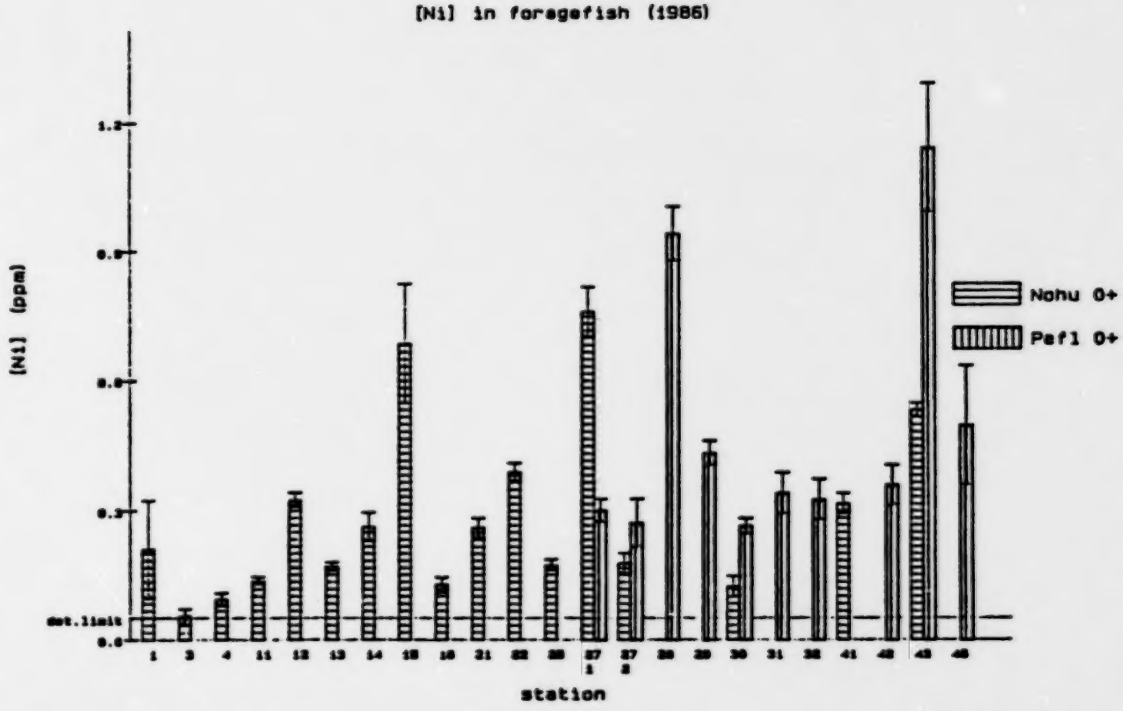

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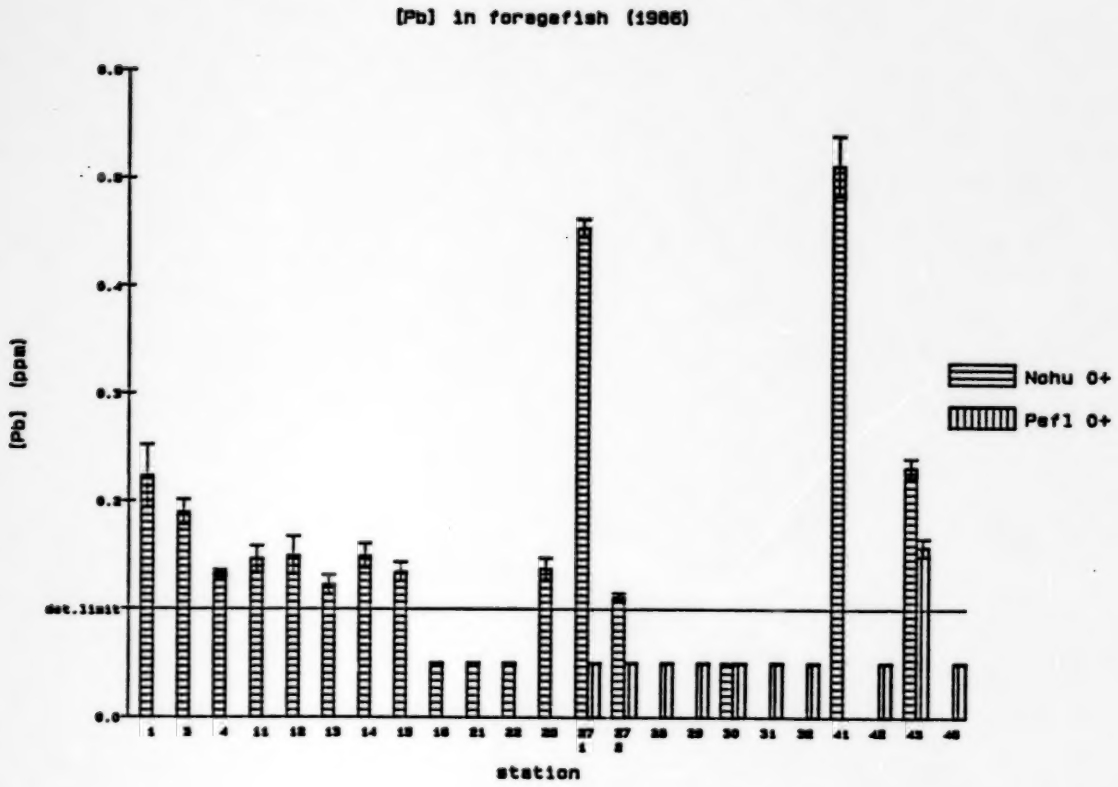

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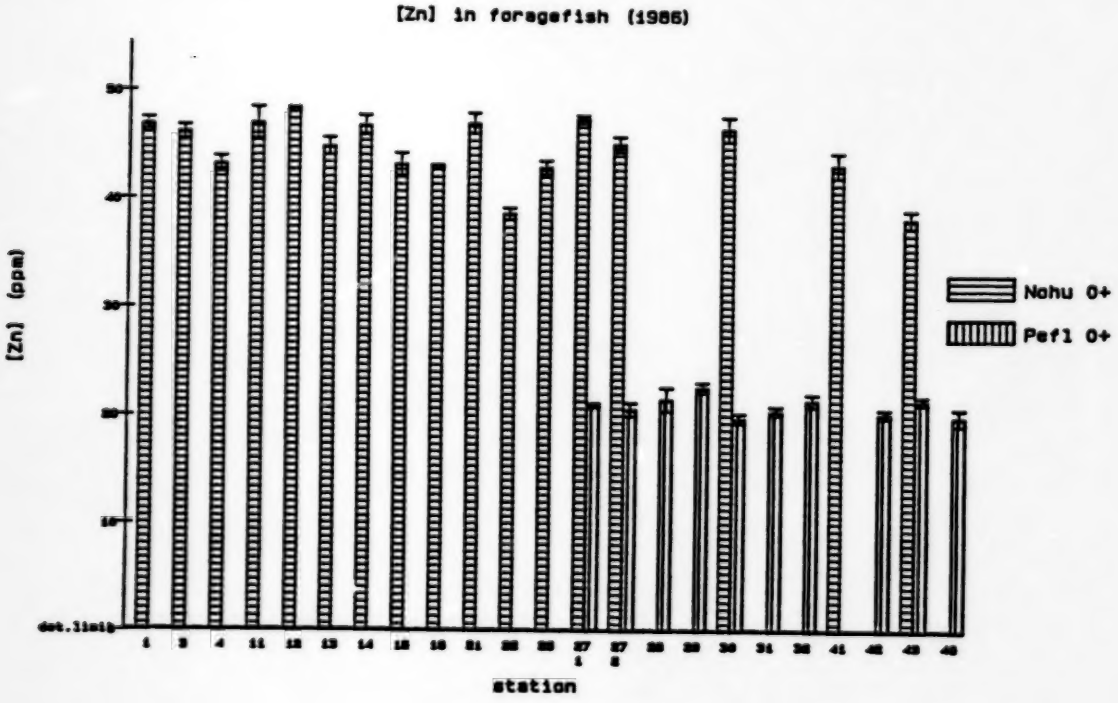

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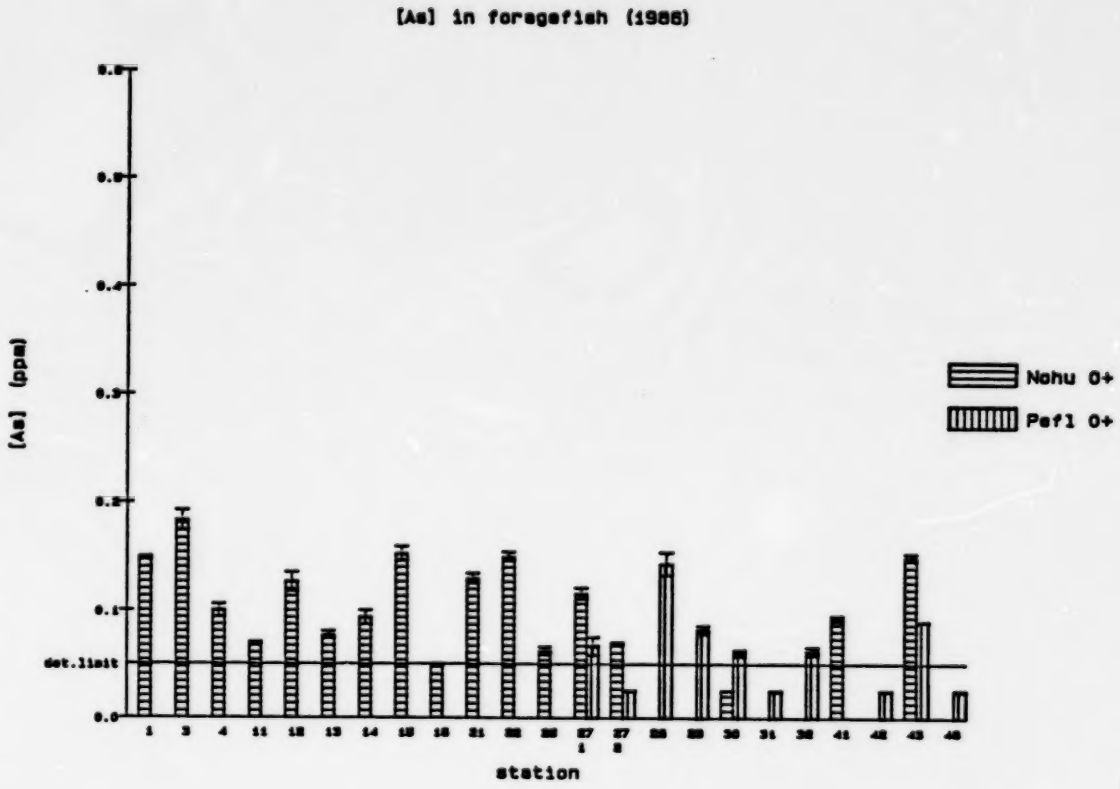

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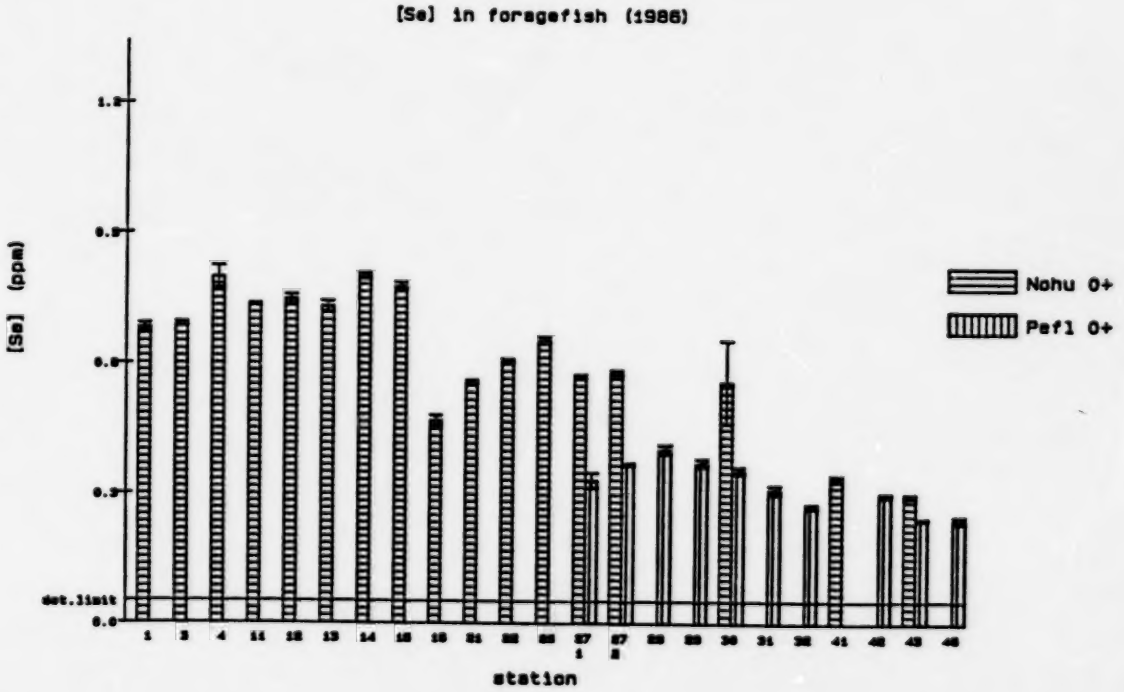

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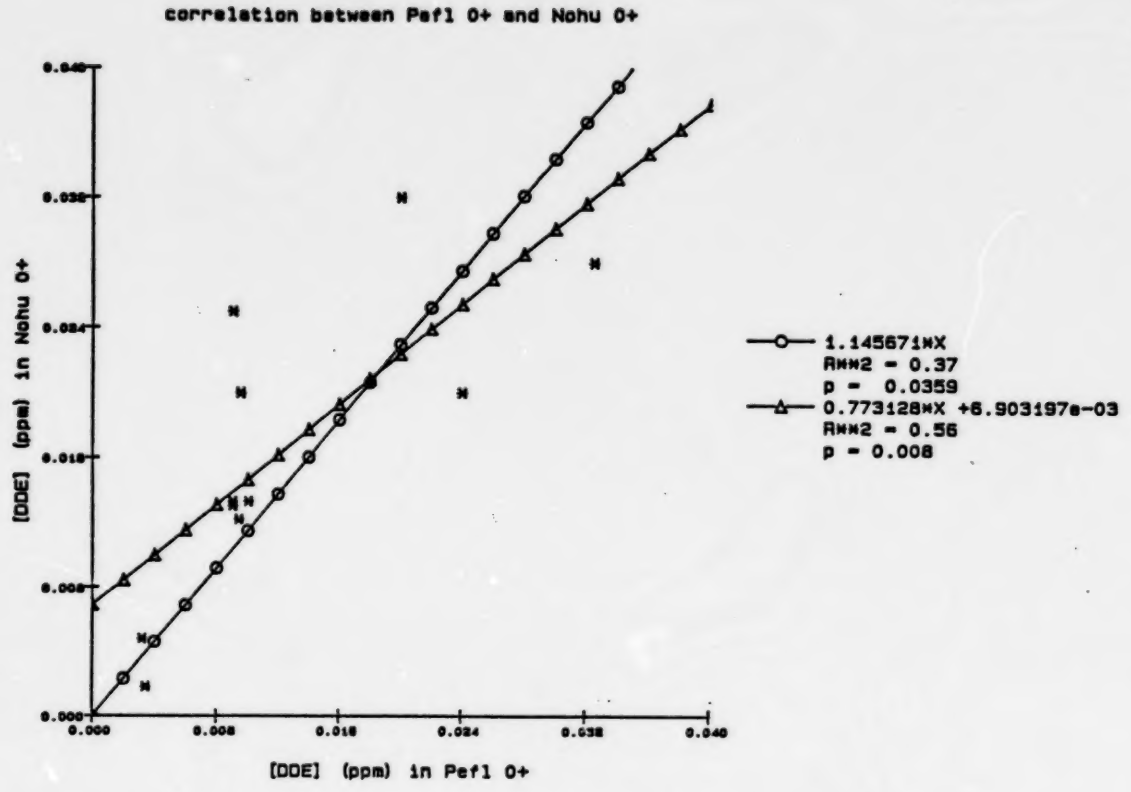

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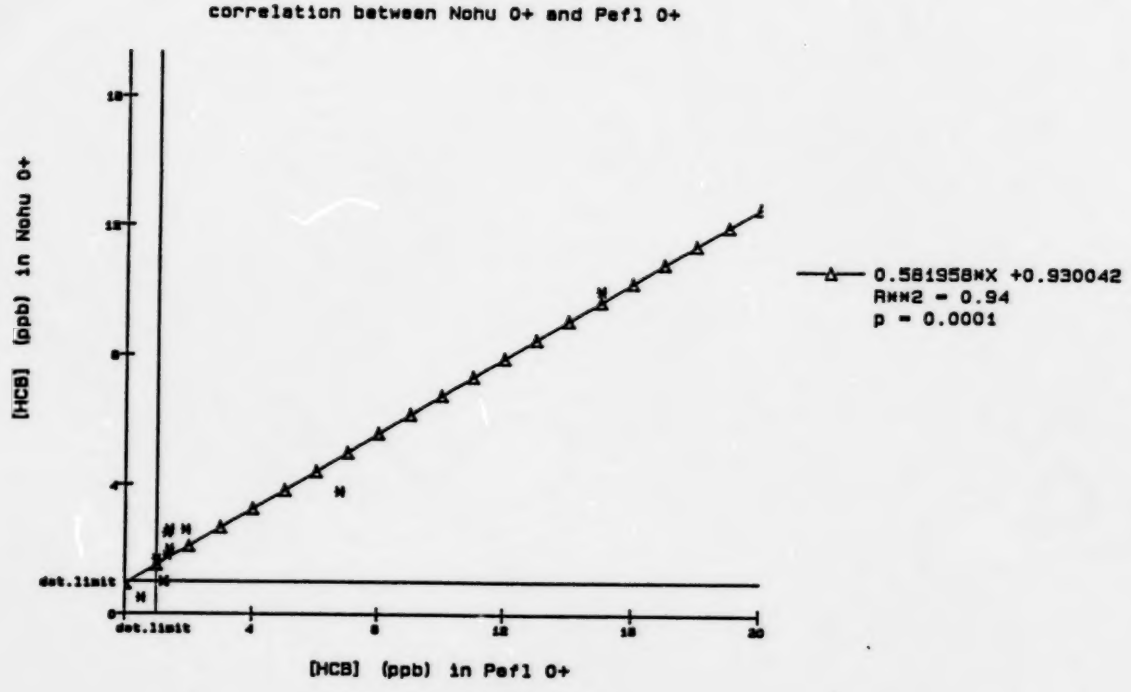

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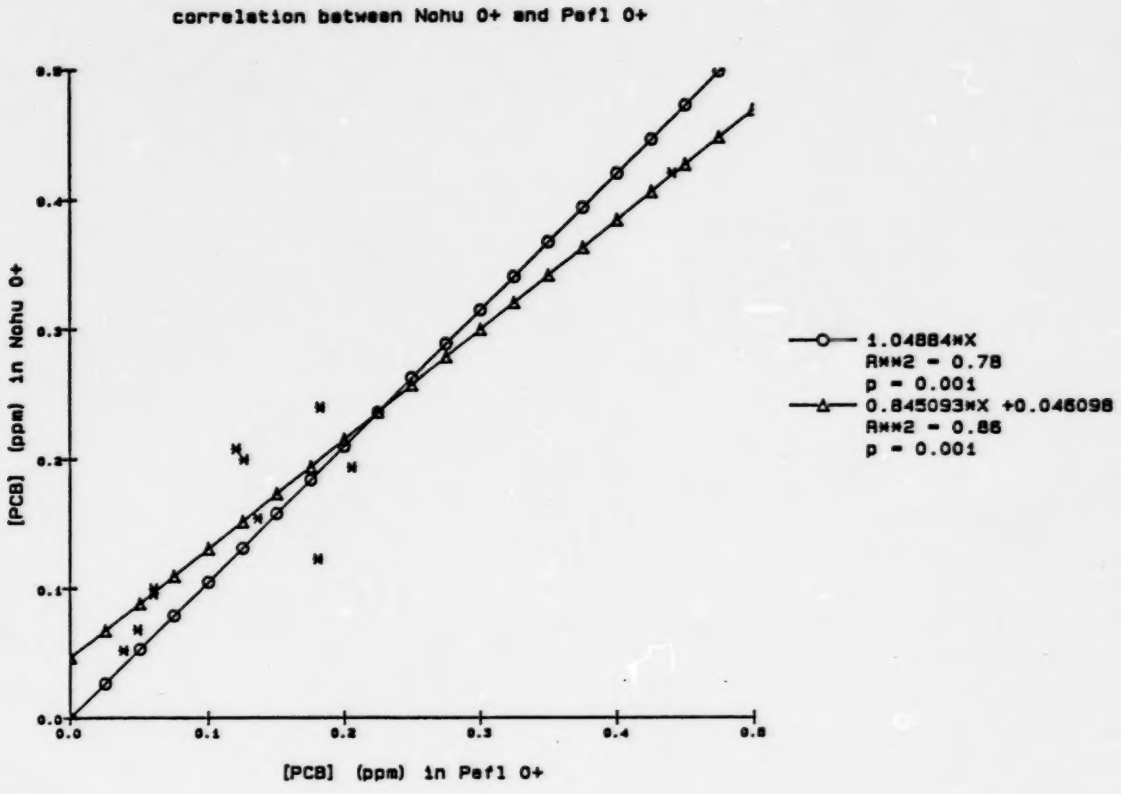

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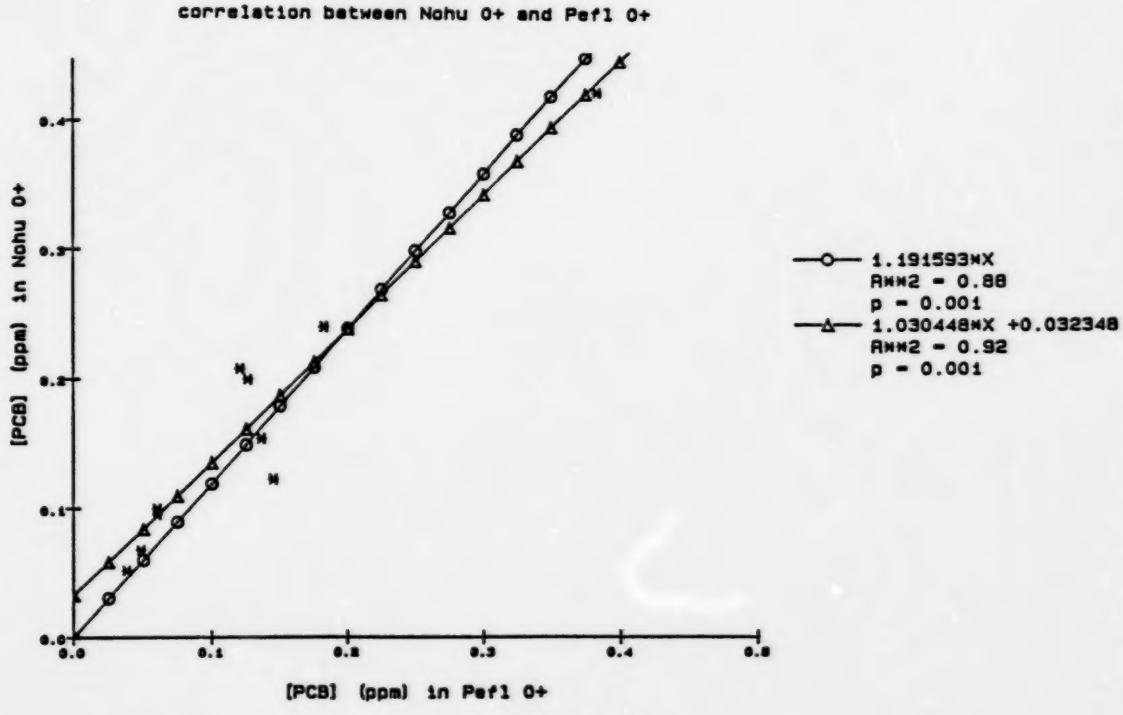

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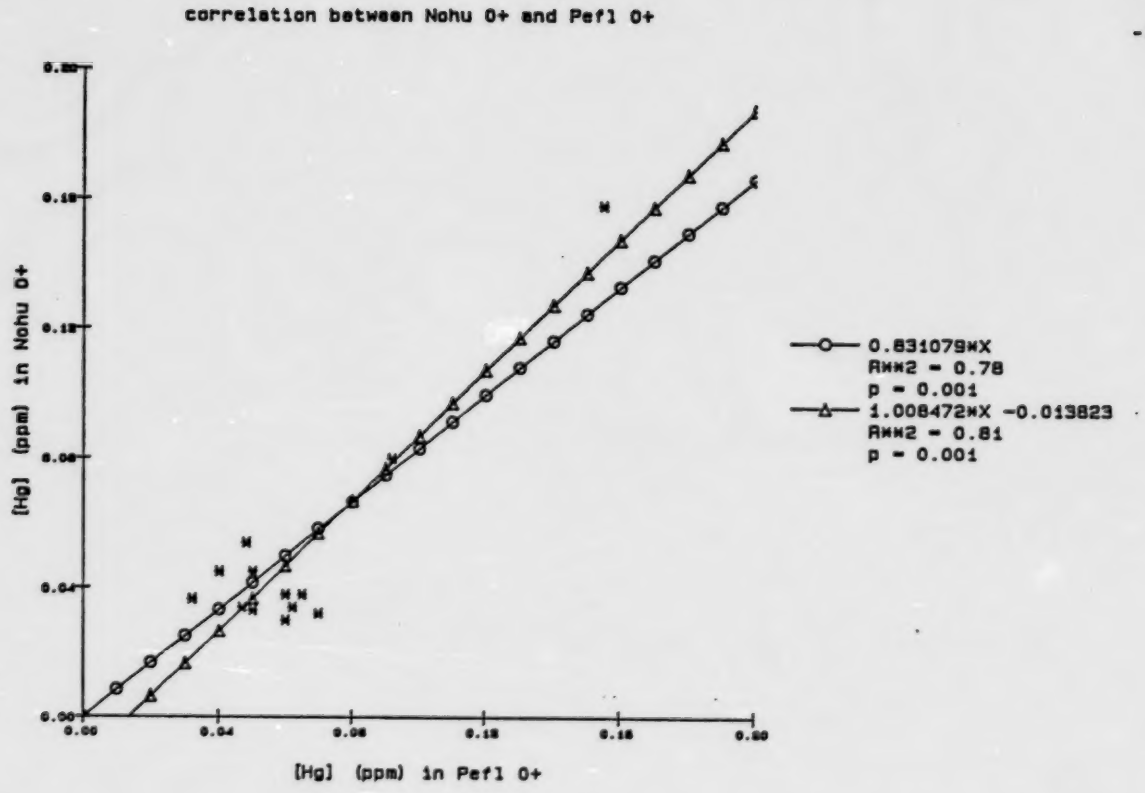

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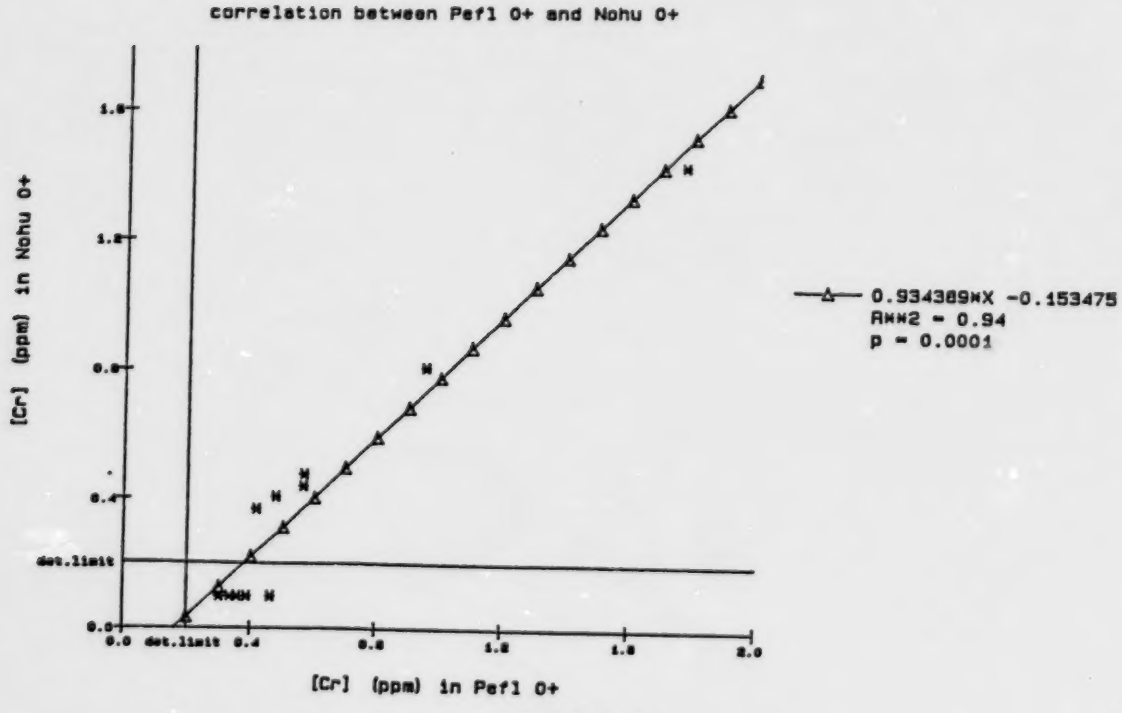

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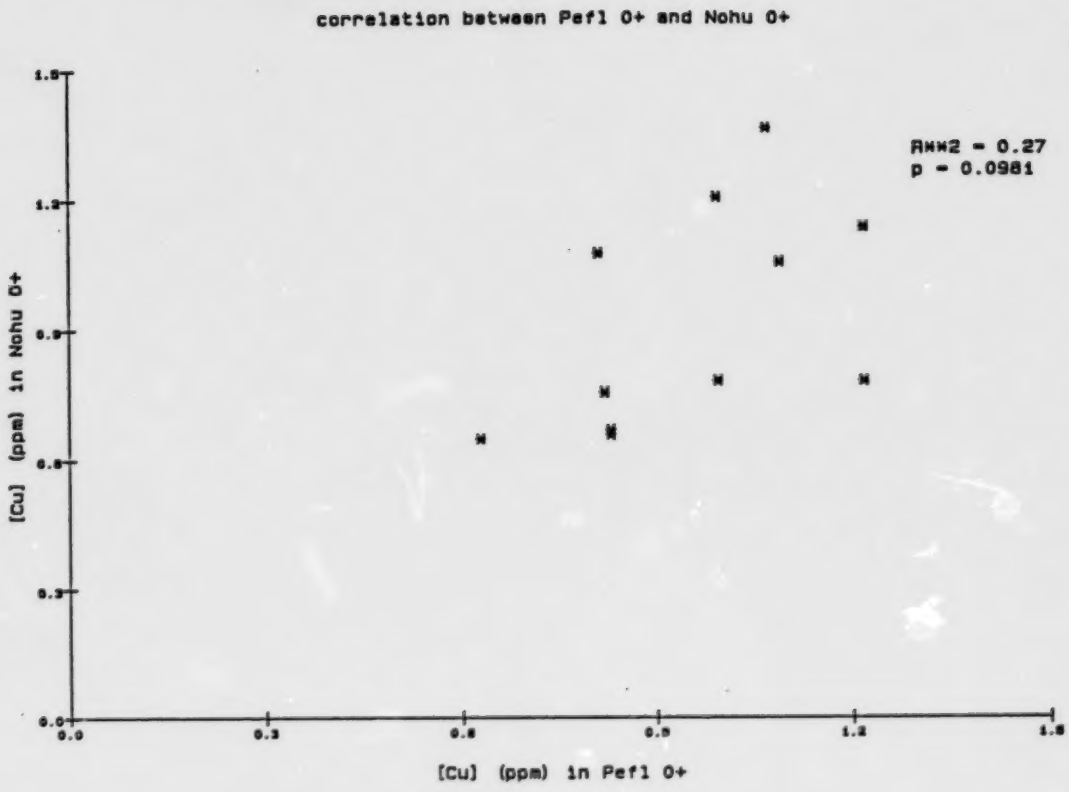

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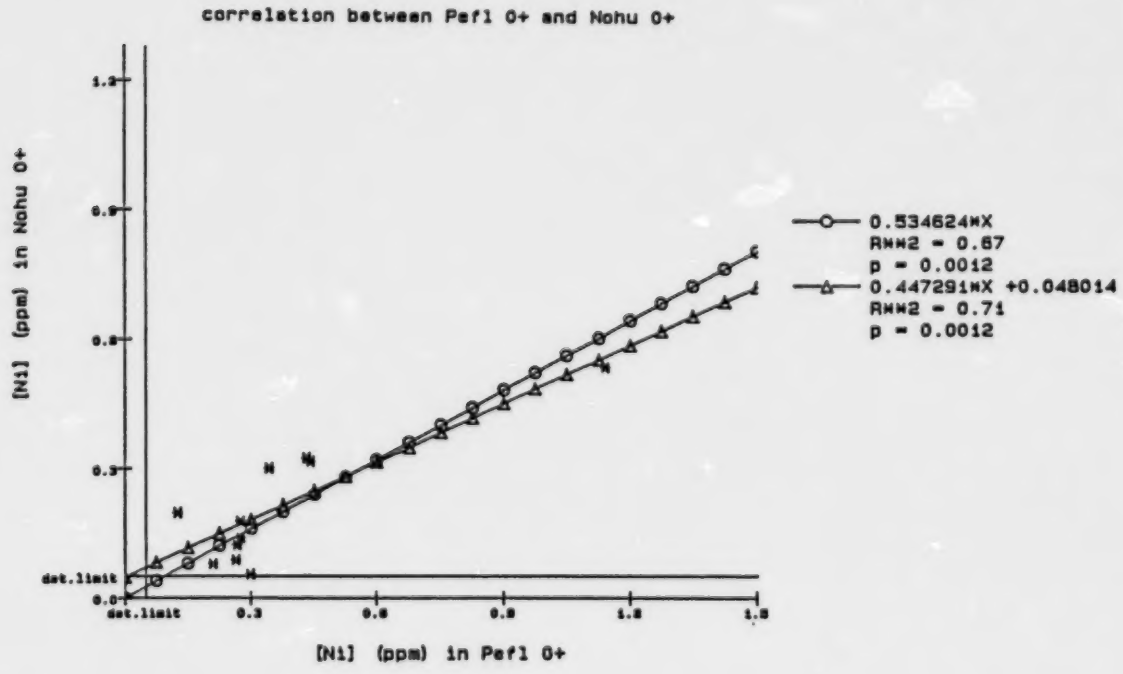

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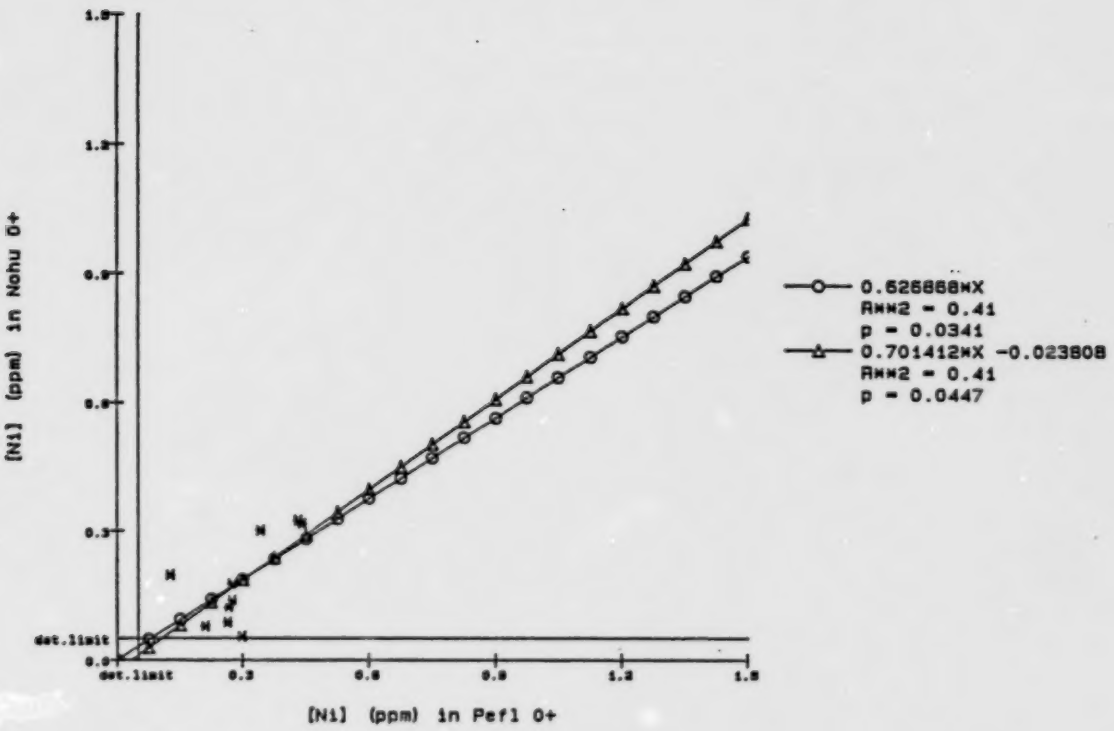

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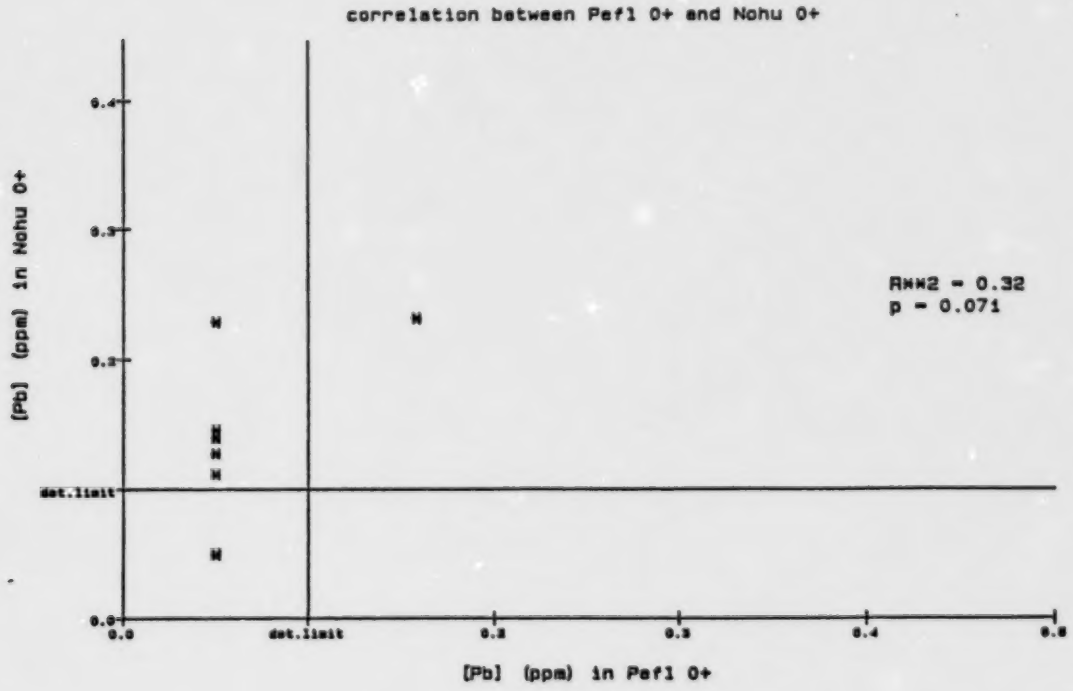

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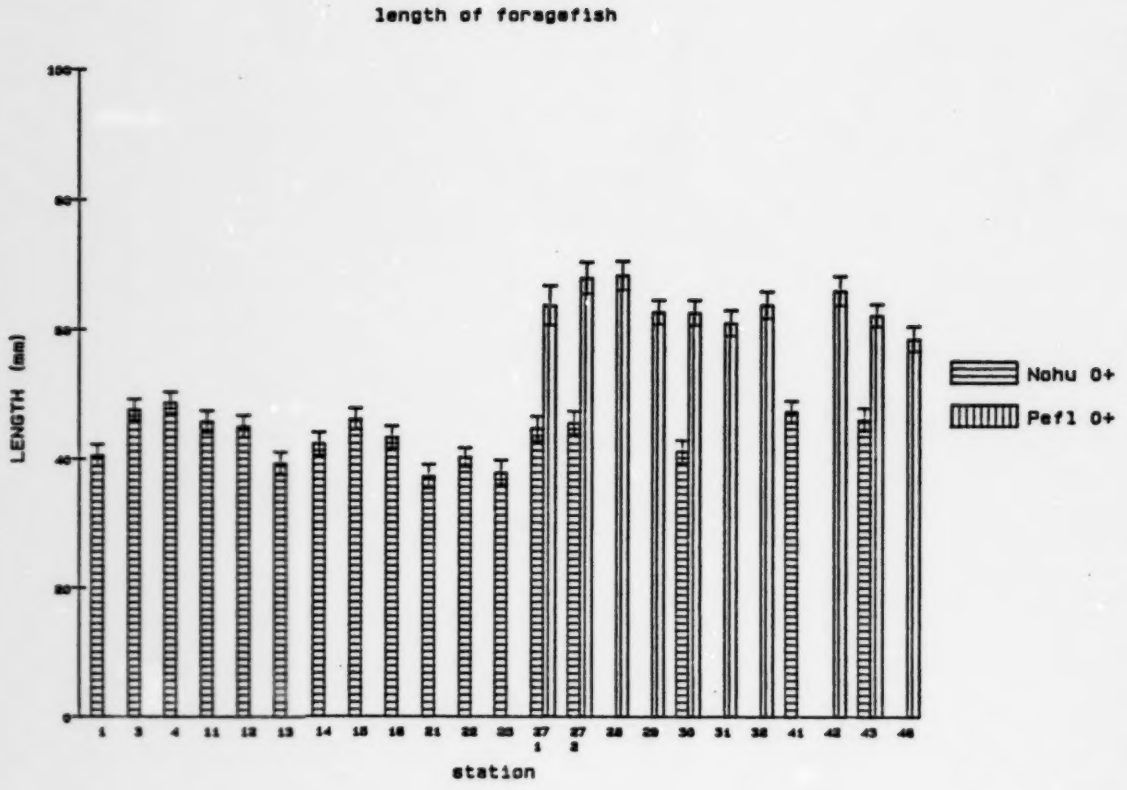

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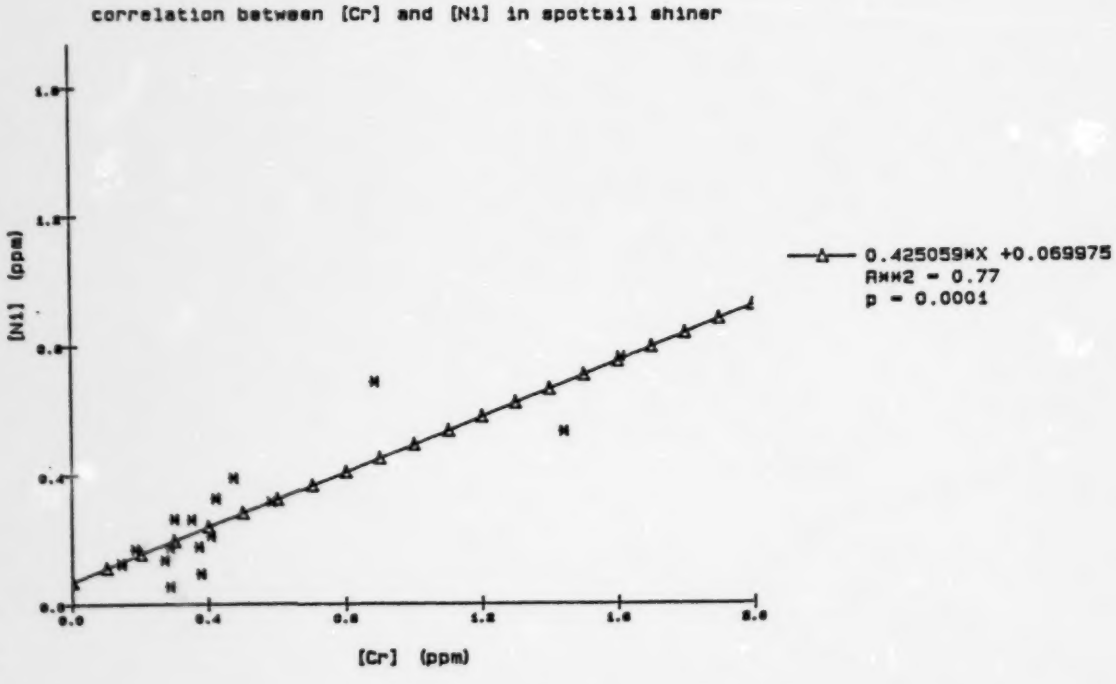

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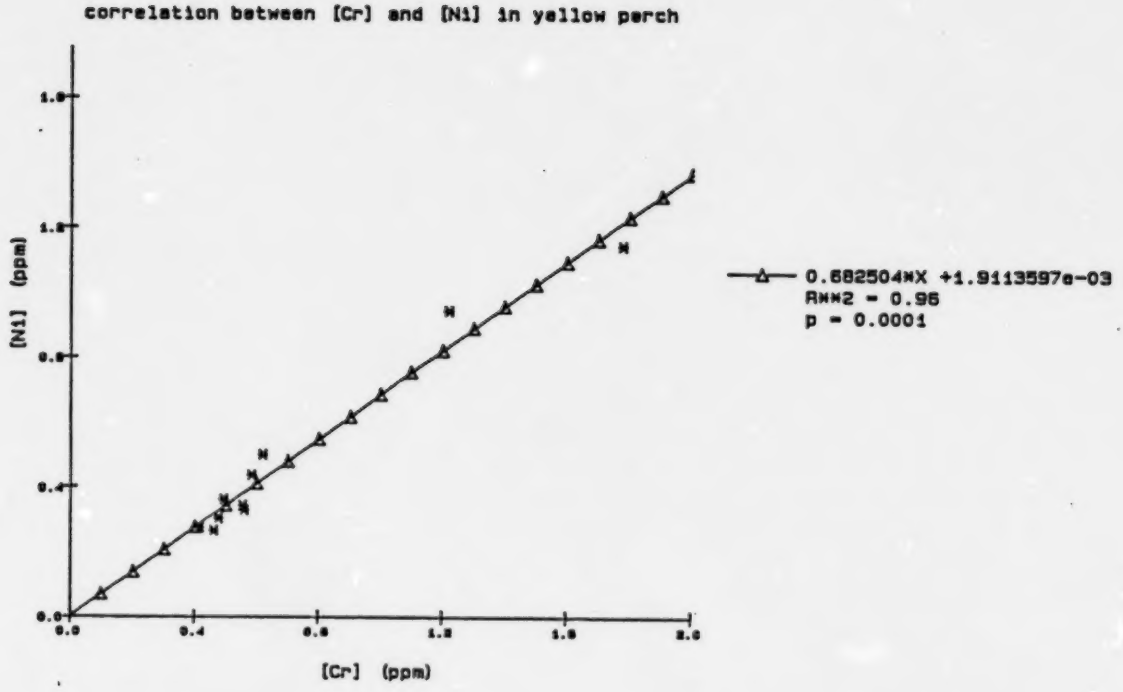

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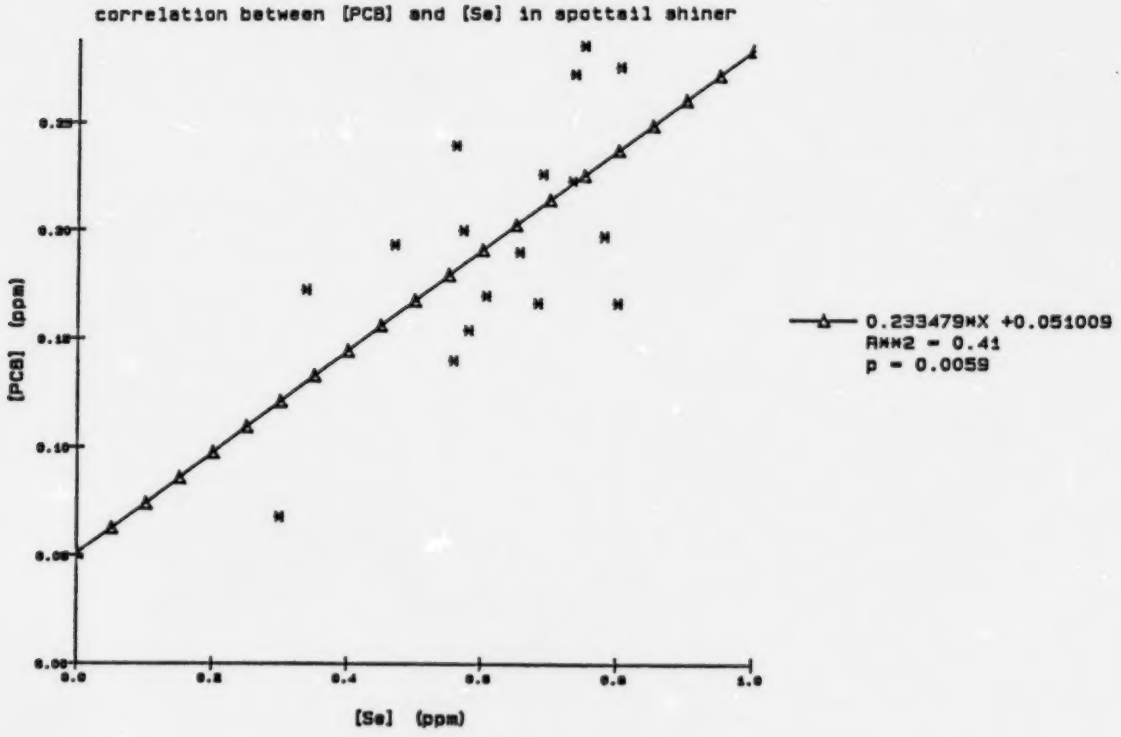

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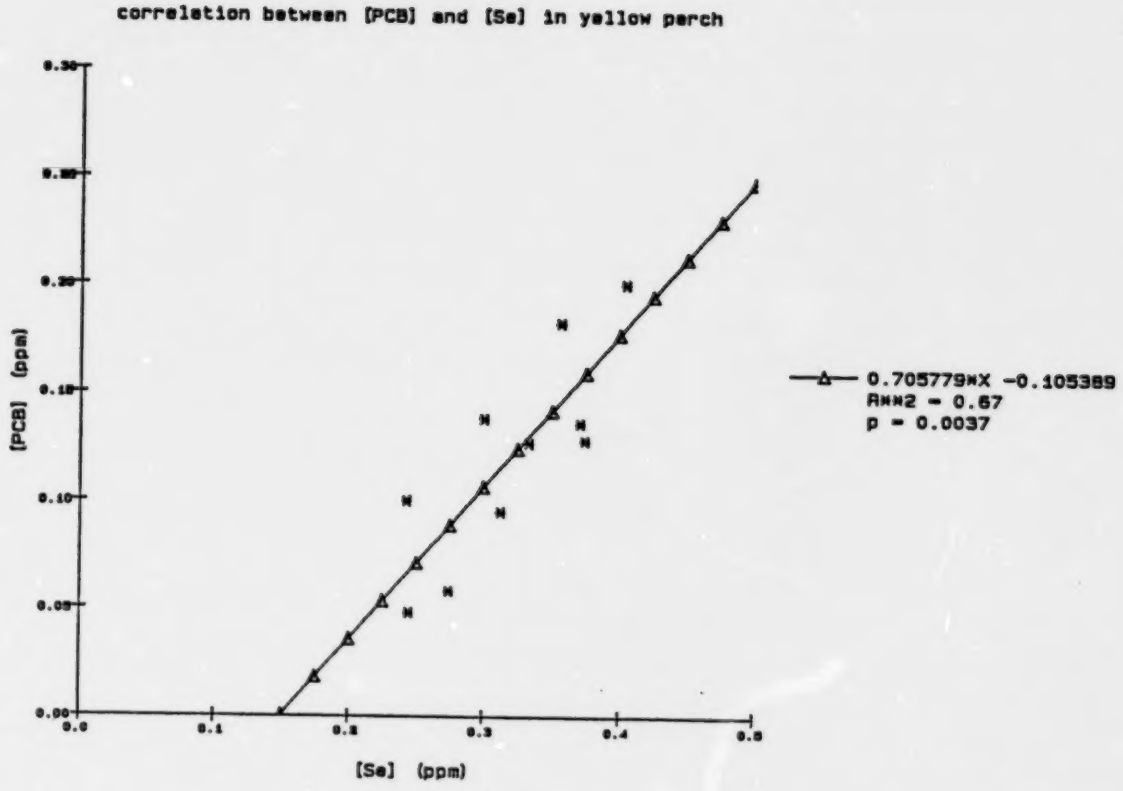

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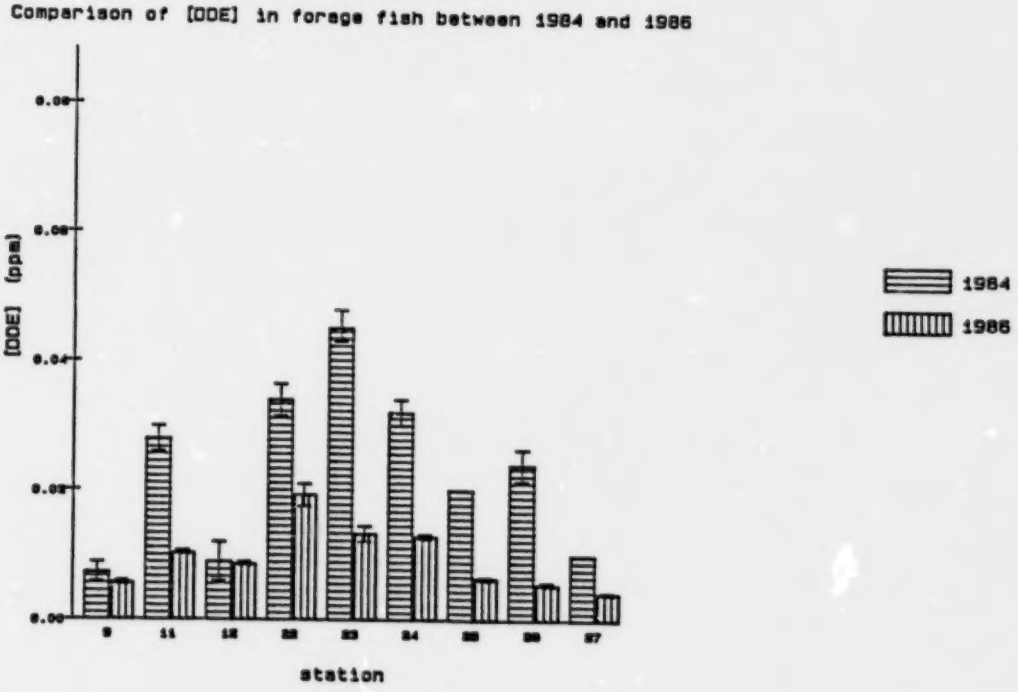

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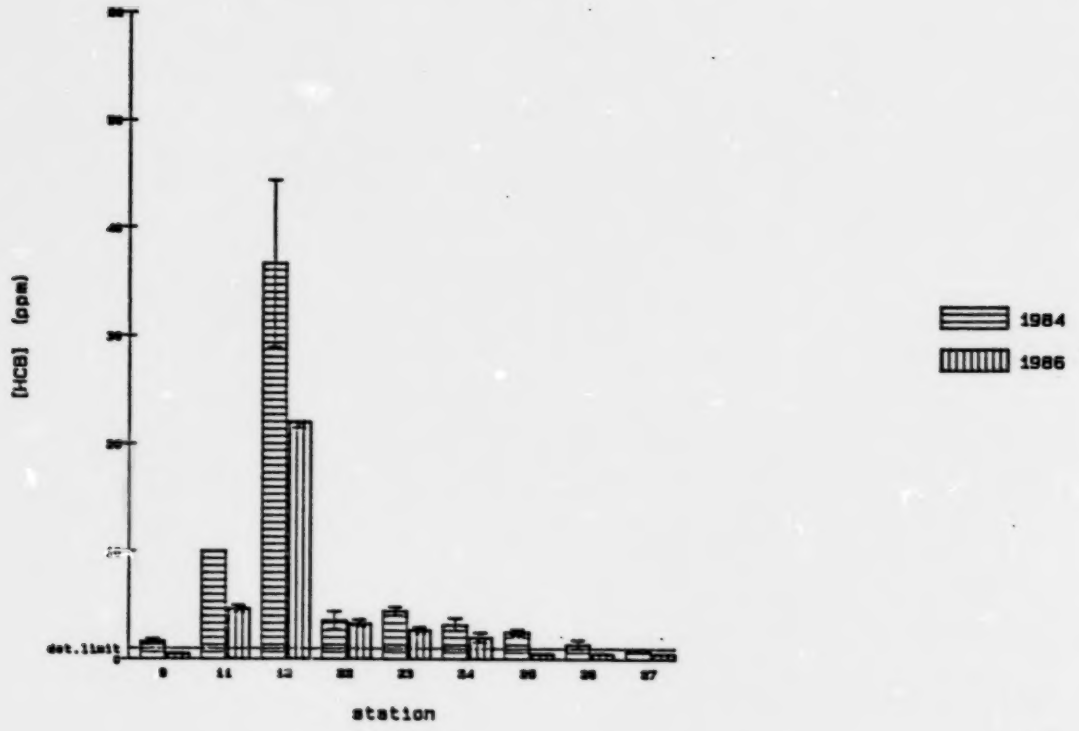

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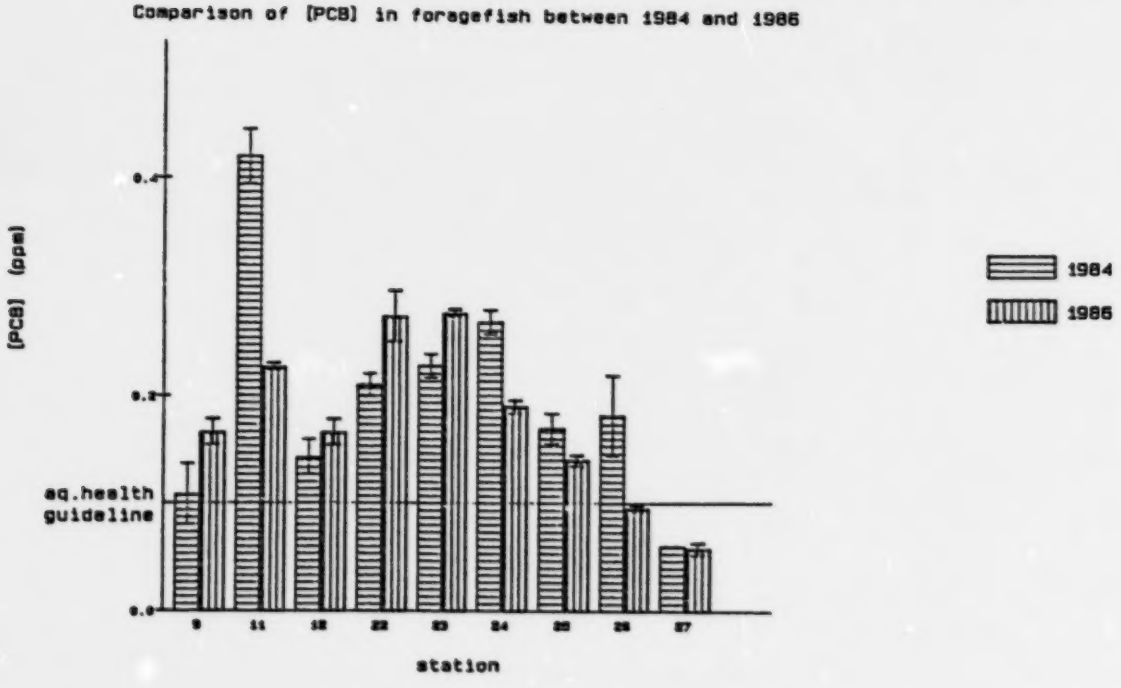

FIGURE 32.

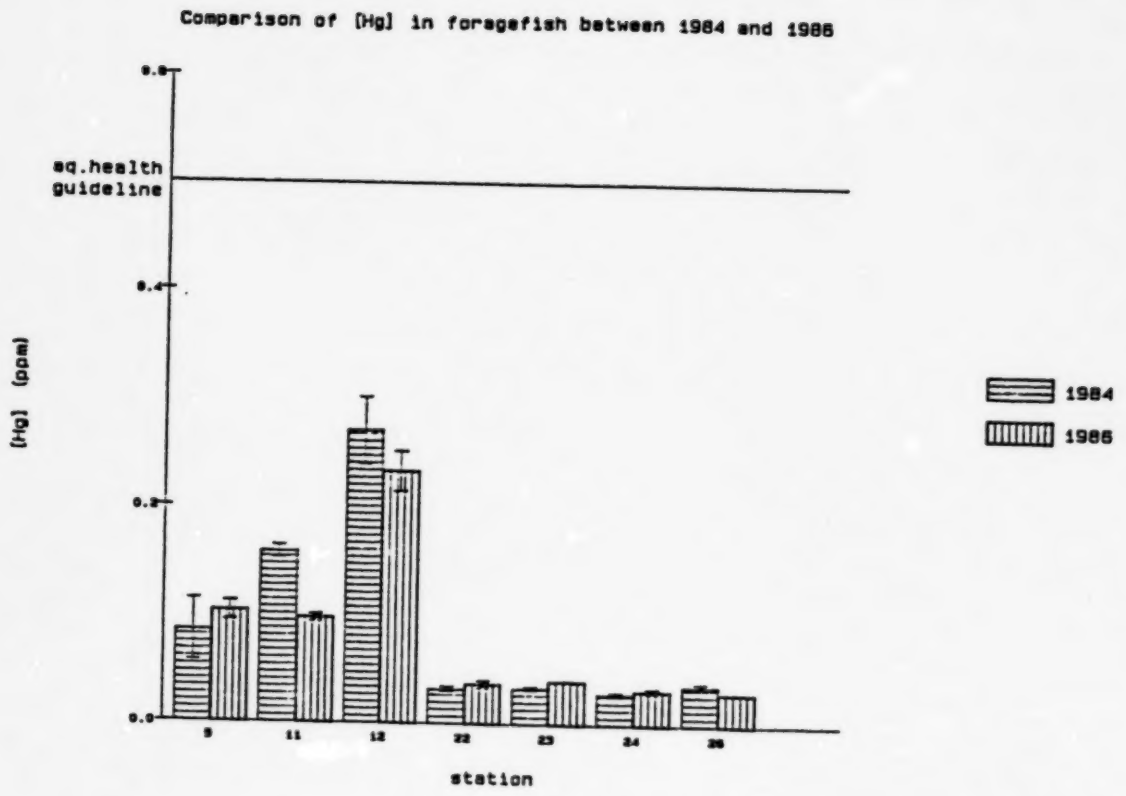

FIGURE 33.

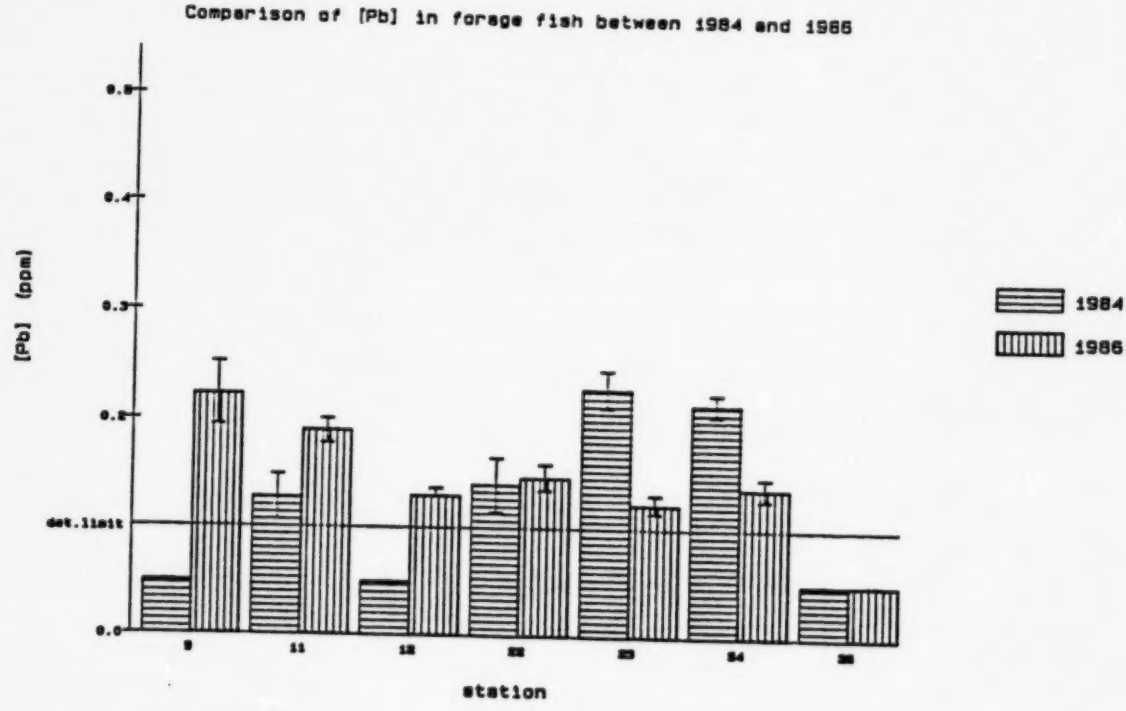

FIGURE 34.

CHAPTER 2

BACKBONE AS A TOOL FOR MEASURING SUBLETHAL EFFECTS ON FISH

2.1 INTRODUCTION

Although chemical analysis of fish or parts of fish provides information on bioavailability, it does not answer the welknown 'so what' question i.e. what is the influence of the presence of toxic chemicals (e.g. organochlorinated compounds and heavy metals) in fish.

PASSINO (1984) gives a good overview of the methods which are available for measuring sublethal effects (stress) in fish. For example the amino levulinic acid dehydratase (ALA-D) activity of lead exposure in fish (HODSON et al., 1984). Another method is biochemical analysis of bone composition, which can be altered by toxic chemicals. MEHRLE & MAYER (1975) report that collagen and hydroxyproline content of backbone in fathead minnows (Pimephales promelas) decreased after continuous exposure to toxaphene. They suggest , that the vitamin C metabolism may have been changed, which affected the collagen synthesis. Perhaps compartition between functions or structures for vitamin C, caused by the toxic chemical, resulted in a functional deficiency of vitamin C in the bone synthesis. This reduction of collagen and hydroxyproline concentrations causes weaker bone structure, which makes the fish more vulnerable to mechanical stress. Therefore mechanical properties are now also analysed in addition to the chemical composition (e.g. HAMILTON et al., 1981a, 1981b, MEHRLE et al., 1982).

To investigate if bone composition is a good monitoring tool for measuring stress in fish under field conditions, yellow perch (Perca flavescens), which was caught during a pilot study in September and October 1984 (GUAY & DANDURAND, 1986), was analysed for backbone composition. Mechanical properties were not investigated, so this study gives only the results of the chemical composition of the backbone.

2.2 MATERIALS AND METHODS

The fish were caught by means of beach seining as part of the pilot study and were immediately frozen on dry ice. Since the objective of the study was evaluating the use of young-of-the-year and yearling fish for bodyburden contents of contaminants, the selection of specimens, especially older yearclasses, for the backbone analysis was rather restricted. The total number of specimens, that the lab could accept was also limited (75 specimens). Furthermore, due to transportation problems, the first batch of fish sent out for backbone analysis arrived thawed out at the lab and were, therefore, unsuitable for analysis. This mishap restricted even more our selection of specimens for a second batch resulting in a rather heterogeneous composition (table 9).

table 9. Amount of fish per station and per age group submitted for backbone analysis.

age	station		
	SL5	SL11	F5
0+	18	-	-
1+	12	10	7
2+	-	10	-
adult	15	5	-

Station SL5 represents a potentially stressed environment, while stations SL11 and F5 are relatively less polluted (station locations in figure 35).

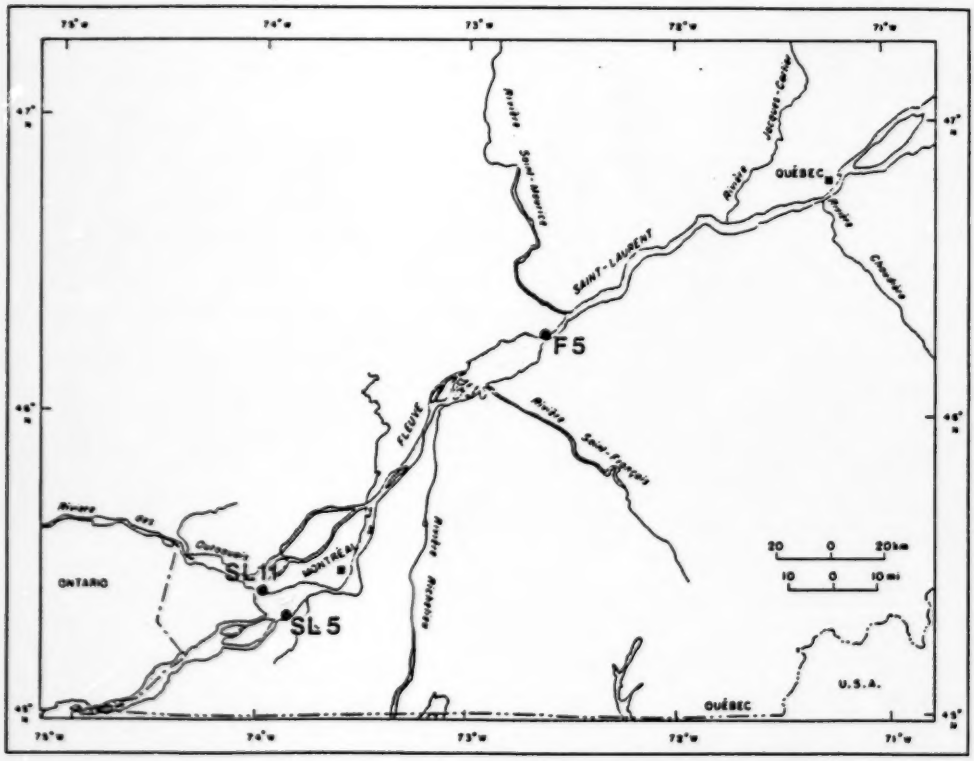

Figure 35. Location of the stations.

Older yearclasses than 0+ were included to determine whether effects may show up at an older age, since exposure time for the 0+ individuals is limited to a maximum of 4-5 months.

The backbone analysis was carried out by the Great Lakes Research Branch, Department of Fisheries and Oceans, Canada Centre for Inland Waters, Burlington (Ont.). From every fish two set of vertebrae were dissected. For the purpose of this report, we use the term duplicate to describe the results of those two sets. Collagen content was measured according to FLANAGAN & NICHELS (1962) and hydroxyproline by the method of WOESSNER (1961). The methods of analysis are described in appendix B.1.

The results of the analysis are tested with an one-way model 2 ANOVA (SOKAL & ROHLF, 1981). The approach is that at first an ANOVA is carried out and only is there is a significant difference between the station-age groups (i.e. an age group at a certain station) partial ANOVA's (ANOVA's which test only a part of the total variance) are carried out to investigate among which groups the difference exist. The partial ANOVA's are orthogonal independent so the result of one does not influence the other. The orthogonal independence between the partial ANOVA's can be verified by summming the sums of squares (SS) of the partial ANOVA's. This is within rounding errors the same as the SS among station-age groups. The descriptive statistics and the one-way ANOVA's were calculated by means of the software package RS1 (ANONYMOUS , 1983). The coefficient of variation was calculated by the method of STEINER (1975), which can be summarized as follows:

coefficient of variation between duplicates =

(SS of ind.repls. - (SS of repl.tot./# of repls.))/
(# of fish per s.a.g.*mean of s.a.g.)

where SS = sum of squares
ind.repls. = individual replicates
# = number
repl.tot. = replicate totals
s.a.g. = station-age group

coefficient of variation within station-age group =

(SS of repl.tot./# of repls. - (mean of s.a.g.**2)*# of fish per s.a.g.)/
(# of fish per s.a.g.*mean of s.a.g.)

2.3 RESULTS

The detailed results of the backbone analysis are presented in appendix B.2. Table 10 lists the descriptive statistics of the different age groups per station (station-age groups).

Table 10. Descriptive statistics for the different station-age groups.
a. collagen(mg)/vertebrae(g)
b. hydroxyproline(µg)/collagen(mg)

a. collagen(mg)/vertebrae(g)

Station:	F5	SL11	SL11	SL11	SL5	SL5	SL5
Age:	1+	1+	2+	ADULT	0+	1+	ADULT
Statistic							
Count (N)	6	10	9	5	18	11	15
Sum	1185	2562	2487	1388	5195	3154	4053
Mean	197.5	256.2	276.3	277.6	288.6	286.6	270.2
S.E. of the mean	43.27	10.71	15.47	5.93	9.74	7.74	10.68
Median	219.75	249	292	272	292	297	258.5
Variance	11235	1146	2154	176.1	1708	659.8	1711
StDev (sd)	106.03	33.86	46.41	13.27	41.33	25.68	41.36
Maximum	394.5	302	326.5	299.5	353.5	316	373.5
Minimum	56	214	195	267	206	246	218
Range	338	88	131.5	32	147.5	70	155.5
Skewness	-0.34	0.31	-1.18	1.53	-0.23	-0.61	1.41
Kurtosis	-2.38	-1.51	0.29	2.02	-0.64	-1.28	2.00

b. hydroxyproline(µg)/collagen(mg)

Station:	F5	SL11	SL11	SL11	SL5	SL5	SL5
Age:	1+	1+	2+	ADULT	0+	1+	ADULT
Statistic							
Count (N)	7	10	10	5	13	12	15
Sum	284.4	456.4	391.3	224.8	506.	499.7	695.8
Mean	40.6	45.6	39.1	44.9	28.1	41.6	46.3
S.E. of the mean	2.66	2.46	3.03	5.07	1.64	3.23	2.69
Median	41.4	42.7	41.92	48.3	26.67	39.82	46.45
Variance	49.59	60.52	92.02	128.39	48.83	125.37	109.16
StDev (sd)	7.04	7.78	9.59	11.35	6.99	11.22	10.44
Maximum	52.45	57.3	50	57.7	41.15	64.35	66.05
Minimum	32.35	31.7	25.25	32	17.5	29.7	26.1
Range	20.2	25.6	24.75	25.7	23.65	34.65	39.95
Skewness	0.54	-0.02	-0.39	-0.24	0.38	1.04	-0.20
Kurtosis	-0.31	-0.20	-1.72	-2.62	-0.61	0.36	-0.05

The mean, standard deviation and 95% confidence limits for collagen and hydroxyproline are graphically presented in figures 36 and 37.

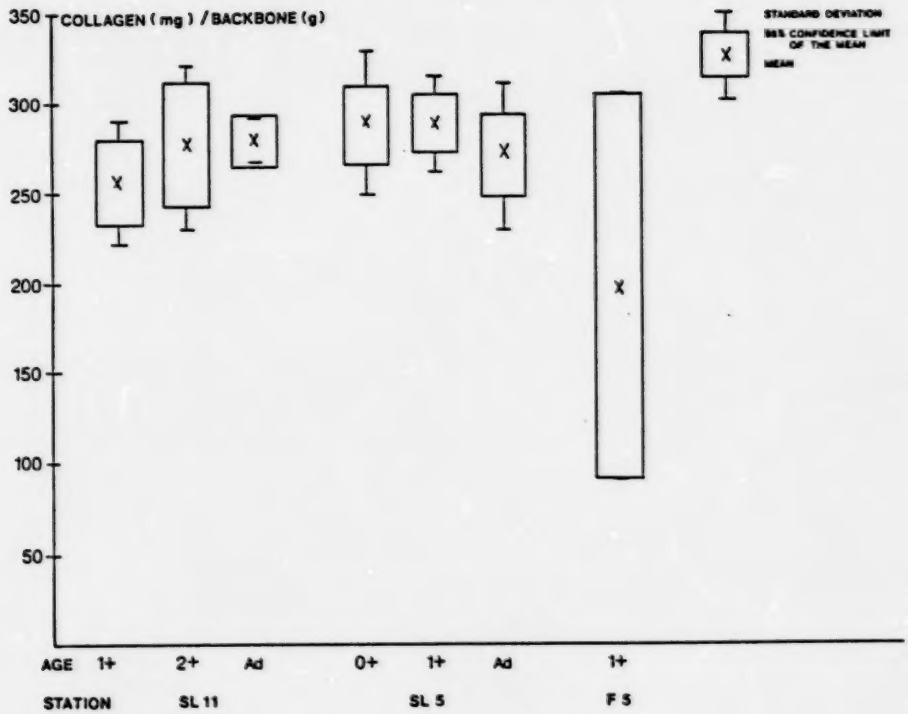

Figure 36. Collagen concentration in the backbone of yellow perch for the different station-age groups.

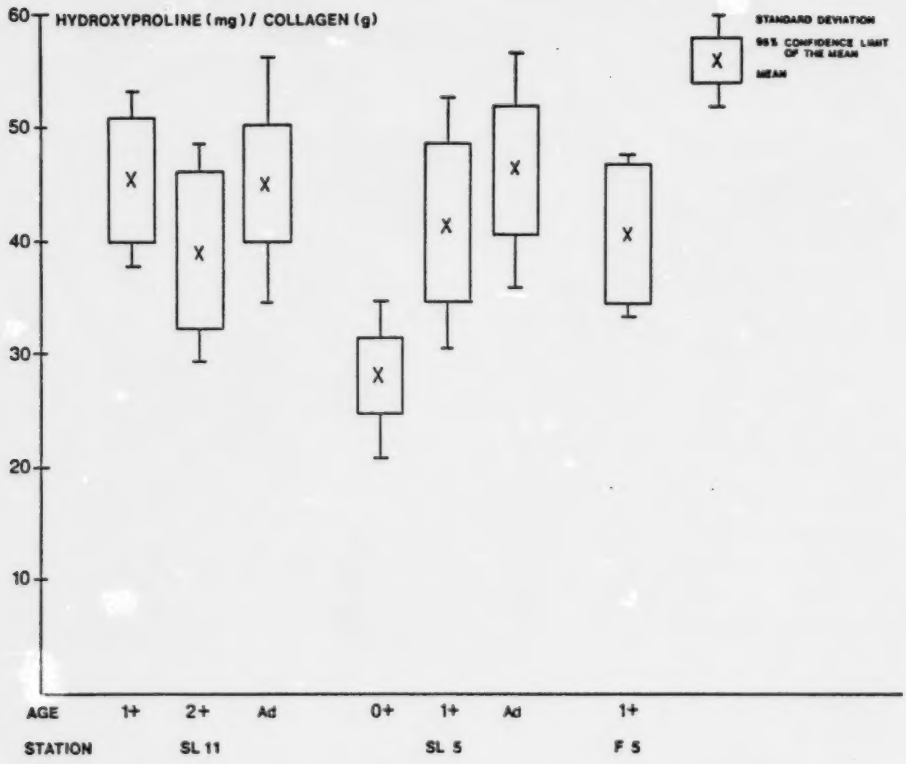

Figure 37. Hydroxyproline concentration in collagen of the backbone of yellow perch for the different station-age groups.

For collagen there was no significant difference between the station-agegroups (table 11a). Station F5 was excluded from the ANOVA, because of unexplainable low collagen concentration in some fish (appendix B.2). Collagen concentrations which are 46-80% lower than normal, compared with the concentrations in other specimens, seems very unlikely, but unfortenately, these results could not be verified.

For hydroxyproline there was a significant difference between the station-age groups (table 11b) which was mainly due to the variation among the age groups within station SL5. No significance difference was found between the reference stations and the more polluted station, between the two reference stations F5 and SL11 and also not among the age groups within station SL11. This result of the ANOVA is clearly visualized in figure 37: the 0+ group at station SL5 is lower than all others, which, for the rest, are almost the same.

Table 11. Analysis of variance classification
a. collagen(mg)/vertebrae(g)
b. hydroxyproline(µg)/collagen(mg)

a. collagen(mg)/vertebrae(g)

	DF	SS	MS=SS/DF	F VALUE	SIG LEVEL
among station-age groups	5	8482.544	1696.709	1.2	0.321
within station-age groups	62	87829.114	1416.599		
total	67	96312.658			

b. hydroxyproline(µg)/collagen(mg)

Source of variance	DF	SS	MS=SS/DF	F VALUE	SIG LEVEL
among station-age groups	6	3630.484	605.581	7.15	0.000
PS and SL11 vs. SL5	1	393.774	393.774	3.22	0.077
PS vs. SL11	1	28.215	28.215	0.35	0.559
among age groups within SL11	2	238.422	119.211	1.39	0.270
among age groups within SL5	2	2973.071	1486.535	16.68	0.000
within station-age groups	70	5930.109	84.716		
total	76	9560.592			

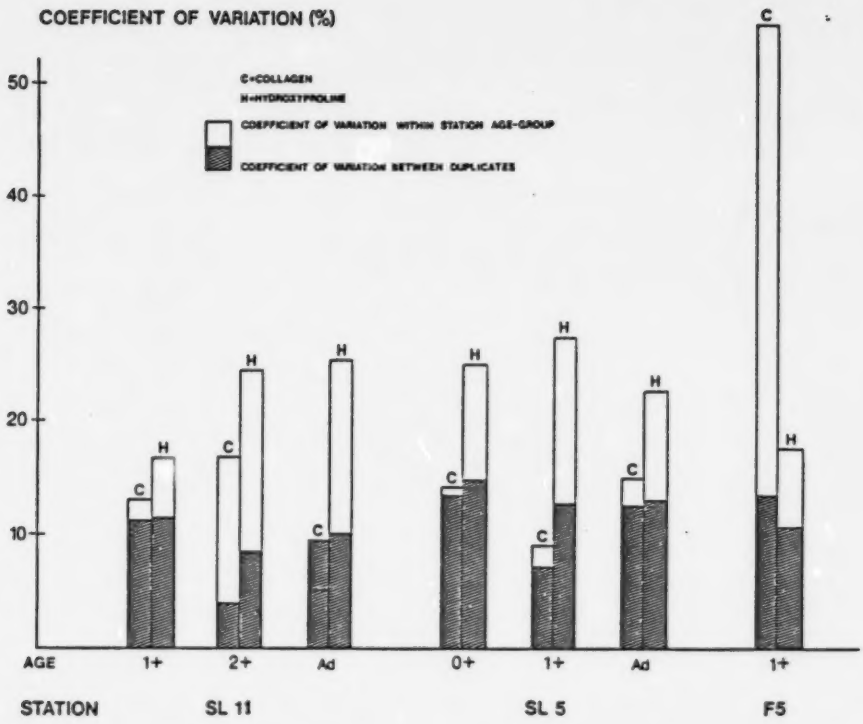

Figure 38. Coefficient of variation of the collagen and hydroxyproline concentration between duplicates within a fish and within a station-age group.

Figure 38 shows the relationship between the coefficient of variation within the fish (between duplicates) and within a station-age group. From figure 38 it is clear that the variation in collagen and hydroxyproline within a fish is an important feature, which could mean, that difference between stations are difficult to detect. If we again exclude station F5 (collagen only) the coefficient of variation within the fish is 3.8-14.4% and within the group 5-17%. For hydroxyproline, the percentages are 8.2-14.4 and 17.0-25.4, respectively.

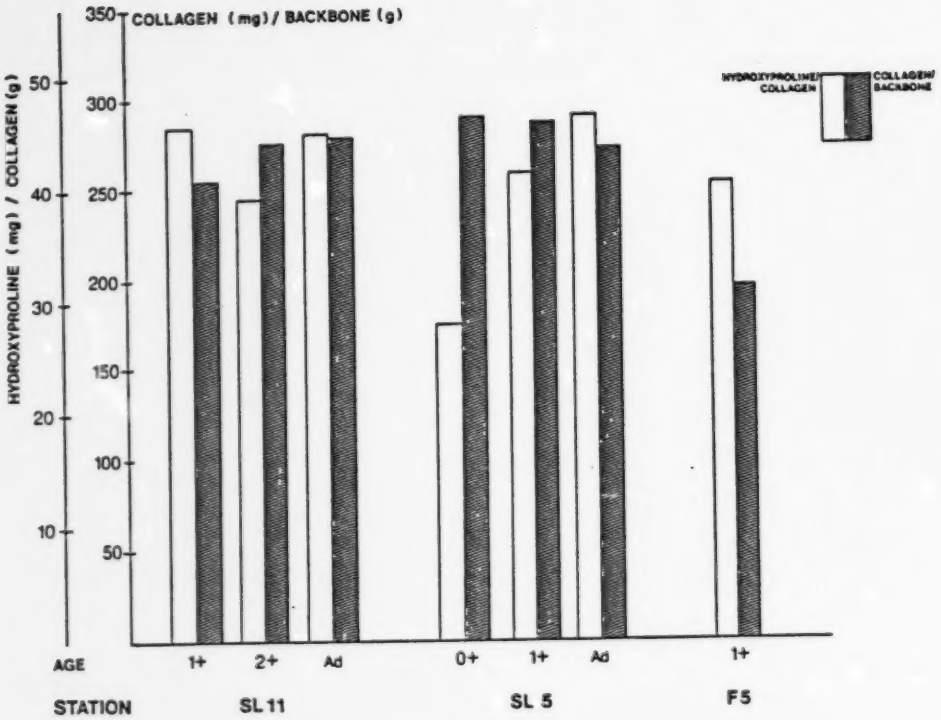

Figure 39. Average hydroxyproline and collagen concentration per station-age group.

Figure 39 displays the average collagen and hydroxyproline concentration per station-age group. This figure shows that there is little resemblance between the patterns of collagen and hydroxyproline concentration in backbone. This could mean that the influence of the contaminants is very little compared with the variation found in a population or that both methods do give contradictory results which makes evaluation of stress by means of bone composition difficult

2.4 DISCUSSION

To investigate whether fish from a more contaminated station have lower collagen and hydroxyproline content in the backbone, hence weaker backbones, than those from a less contaminated station, yellow perch of different ages from the stations F5, SL5 and SL11 were

analysed. In table 12 are presented the concentrations of toxic chemicals in young (young-of-the-year and yearling) yellow perch and spottail shiner which were caught during the same pilot study in 1984. These results confirm that station SL5 is definitely the more polluted station especially for PCB's and Hg.

Table 12. Bodyburden content (ppm) of toxic chemicals in young-of-the-year spottail shiner (Nohu), young-of-the-year (Pef1) and yearling (Pef1+) yellow perch at the different stations.

station:	E5	E5	E5	SL5	SL5	SL5	SL11	SL11	SL11
fish:	Nohu	Pef1	Pef1+	Nohu	Pef1	Pef1+	Nohu	Pef1	Pef1+
chemical									
DDE	.02	.024	.026	.032	.019	.022	.009		.03
HCB	.002	.001	.001	.004	.005	.002	.001		.002
PCB	.12	.18	.21	.19	.29	.36	.07		.15
Hg		.036	.072	.126	.215	.237	.036	.065	.094

We did not find, however, lower concentrations of collagen and hydroxyproline at station SL5 and the collagen and hydroxyproline concentration did not show the same patterns over the stations. Only the hydroxyproline concentration of age group 0+ at station SL5 is lower than the others. This could however also be an age effect, which could not be verified, because no 0+ group were analysed for the reference stations.

At the moment the analysis of backbone is still in an experimental stage for use under field conditions. Even Mehrle, Mayer and Hamilton, who already worked for years (mostly under lab conditions) on this subject, obtain contradictory results between different fish species (e.g. compare MEHRLE & MAYER (1975) with HAMILTON et al. (1981b)).

Mechanical properties (e.g. elasticity, toughness, ultimate strain) have been studied by the same authors (HAMILTON et al., 1981a). It seems that mechanical properties are more sensitive to environmental stress than chemical characteristics (MEHRLE & MAYER, 1982). The results between different fish species for mechanical properties were, however, also found to be contradictory (HAMILTON et al. 1981b).

The above authors worked mostly with one or two year old fish. It has been suggested that backbone analysis should be carried out on older fish, because a change in the backbone composition, which can be reflected by mechanical properties, needs some time to manifest itself (M.WHITTLE pers. com.). This means that young-of-the-year fish may

be analysed for toxic chemicals while older fish should be used for detection of sublethal effects.

If, however, sublethal effects of toxic chemicals, reflected by a change in the composition of the backbone, are to be detected, they will have to be fairly large, because the within station-age group variation is about 10% for the collagen and 20% for the hydroxyproline concentration. Sensitivity of the method might be increased by analysing a large number of fishes per group, which would, however, makes it less useful as a monitoring tool in the field.

2.5 CONCLUSIONS AND RECOMMENDATIONS

It is very difficult to draw conclusions based on these results of the backbone analysis, which are certainly influenced by the composition of the batch. The limited amount and the heterogeneity of the samples are , without doubt, some of the major causes preventing a good evaluation of the method. Furthermore the within station variation was larger than the between station variation. Only age group 0+ of station SL5 had a significant lower concentration of hydroxyproline in comparison with the other station-age groups. As mentioned before this could be an age effect.

From the literature it is not clear if the method is already suitable as a monitoring tool in the field. The work of Mehrle, Mayer and Hamilton was mostly caried out under lab-conditions. At the moment is seems to be only a matter of time before the method is ready for use in the field. Especially when coupled with X-rays for screening populations for deformities of the backbone, it could be a valuable tool for measuring stress of toxic chemicals on fish (M.WHITTLE pers.com.). Our batch of yellow perch was also screened by X-rays for deformities of the backbone and there was no correlation found with lower collagen and hydroxyproline concentrations.

If a similar study is carried in the future there should be a more homogeneous and, if possible, a larger batch analysed then was the case with this study. The fish should then also be analysed for mechanical properties because this could be a more sensitive tool as the chemical parameters. It is also possible that the pollution has to be more severe to detect differences with a reference station. MAUCK et al. (1978) report a lab study with Aroclor 1254. They find a decrease in the hydroxyproline concentration in young fish at a waterconcentration of 3.1 ug/l. The observed bioconcentration factor was 40.000 to 47.000. This means the fish contained at least 124 ppm of Aroclor 1254 as bodyburden, which is at least 300 times higher than in our study. These were 'shortterm' effects (exposure time 118 days) and therefore sublethal effects could probably be detected at lower concentrations after a longer exposure time. They also tested only one compound, while in nature we find many different toxic chemicals together.

One of the major problems in the future could, however, become the extrapolating of results from a study on one fish species to other species. This means, that one species could have altered bone composition while another species is normal, although they have the same bodyburden of toxic chemicals in and were caught at the same place and time.

2.6 REFERENCES

ANONYMOUS, 1983. RS/1 user's guide. Book 2: graphics and statistics. BBN software products corporation. pp 195-229.

FLANAGAN, B. & G.NICHOLS Jr., 1962. Metabolic studies of bone in vitro (collagen biosynthesis by surviving bone fragments in vitro). J. of Biol. Chem. 237: 3686-3692.

GUAY,G. & J. DANDURAND, 1986. Utilisation des jeunes poissons de l'année comme bioindicateur des substances toxiques dans le fleuve Saint-Laurent (tronçon fluvial: Cornwall-Portneuf). Final report presented to the Department of Supply and Services and Environment Canada, Inland Waters Directorate. 132 p.

HAMILTON, S.J., P.M.MEHRLE & F.L.MAYER, 1981a. Method to evaluate mechanical properties of bone in fish. Trans. of the Amer. Fish. Soc. 110: 708-717

HAMILTON, S.J., P.M.MEHRLE & F.L.MAYER, 1981b. Mechanical properties of bone in in channel catfish as affected by vitamin c and toxaphene. Trans. of the Amer. Fish. Soc. 110: 708-717

HODSON, P.V., B.R.BLUNT & D.M.WHITTLE, 1984. Monitoring lead exposure of fish. In: CAIRNS, V.W., P.V.HODSON & J.O.NRIAGU. Contaminants effects on fisheries. Advances in environmental science and technology vol. 16. John Wiley & sons. pp. 87-98.

MAUCK, W.L., P.M.MEHRLE, F.L.MAYER, 1978. Effects of the polychlorinated biphenyl Aroclor 1254 on growth, survival and bone development in brook trout (Salvelinus fontinalis). J. Fish. Res. Board Can 35: 1084-1088.

MEHRLE, P.M., T.A.HAINES, S.J.HAMILTON, J.L.LUDKE, F.L.MAYER & M.A.RIBICK, 1982. Relationship between body contaminants and bone development in East-coast striped bass. Trans. of the Amer. Fish. Soc. 111: 231-241.

MEHRLE, P.M. & F.L.MAYER, 1975. Toxaphene effects on growth and bone composition fathead minnows, Pimephales promelas. J. Fish. Res. Board Can. 32: 593-598.

PASSINO, D.R.M., 1984. Biochemical indicators of stress in fishes: an overview. In: CAIRNS, V.W., P.V.HODSON & J.O.NRIAGU. Contaminants effects on fisheries. Advances in environmental science and technology vol. 16. John Wiley & sons. pp. 37-50.

SOKAL, R.R. & F.J.ROHLF, 1981. Biometry. Second edition. W.H.Freeman and company. pp. 232-242.

STEINER, E.H., 1975. Planning and analysis of results of collaborative tests. Statistical manual of the asociation of official analytical chemists. pp. 76-80.

WOESSNER, J.F, Jr., 1961. The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. Arch. Biochem. Biophy. 93: 440-447.

APPENDIX A

A.1 LIST OF SPECIES OF FISH CAUGHT DURING THE 1986-STUDY OF TOXIC SUBSTANCES IN FISH IN THE ST.LAWRENCE RIVER.

Names in accordance to SCOTT & CROSSMAN (1973). Source: GUAY (1986).

SCIENTIFIC NAME	ABBREVIATION	ENGLISH NAME	FRENCH NAME
Amia calva Linnaeus	Amca	bowfin	poisson-castor
Alosa pseudoharengus (Wilson)	Alps	alewife	gaspareau
Osmerus mordax (Mitchill)	Osmo	rainbow smelt	eperlan arc-en-ciel
Hiodon tergisus Lesueur	Hite	mooneye	laquaiche argentee
Esox lucius Linnaeus	Eslu	northern pike	grand brochet
Esox masquinongy Mitchill	Esma	muskellunge	maskinonge
Cyprinidae	Cypr	minnow spp.	mene spp.
Cyprinus carpio Linnaeus	Cyca	carp	carpe
Exoglossum maxillingua (Lesueur)	Exma	cutlips minnow	bec-de-lievre
Notemigonus crysoleucas (Mitchill)	Nocr	golden shiner	chatte de l'est
Notropis atherinoides Rafinesque	Noat	emerald shiner	mene emeraude
Notropis bifrenatus (Cope)	Nobi	bridled shiner	mene d'herbe
Notropis heterodon (Cope)	Noha	blackchin shiner	menton noir
Notropis heterolepis Eigenmann and Eigenmann	Nole	blacknose shiner	museau noir
Notropis hudsonius (Clinton)	Nohu	spottail shiner	queue a tache noire
Pimephales notatus (Rafinesque)	Pino	bluntnose minnow	ventre-pourri
Pimephales promelas Rafinesque	Pipr	fathead minnow	tete-de-boule
Catostomidae	Casp	sucker spp.	meunier spp.
Catostomus commersoni (Lacepede)	Caco	white sucker	meunier noir
Moxostoma anisurum (Rafinesque)	Moan	silver redhorse	suceur blanc
Moxostoma macrolepidotum (Lesueur)	Momm	shorthead redhorse	suceur rouge
Ictalurus nebulosus (Lesueur)	Icne	brown bullhead	barbotte brune
Anguilla rostrata (Lesueur)	Anro	American eel	anguille d'Amerique
Fundulus diaphanus (Lesueur)	Fudi	banded killifish	fondule barre
Apeltes quadracus (Mitchill)	Apqu	fourspine stickleback	epinoche a quatre epines
Culaea inconstans (Kirtland)	Cuin	brook stickleback	epinoche a cinq epines
Percopsis omiscomaycus (Walbaum)	Peom	trout-perch	omisco
Morone americana (Gmelin)	Moam	white perch	bar-peche
Ambloplites rupestris (Rafinesque)	Amru	rock bass	crapet de roche
Lepomis gibbosus (Linnaeus)	Legi	pumpkinseed	crapet-soleil
Micropterus dolomieui Lacepede	Mido	smallmouth bass	achigan a petite bouche
Micropterus salmoides (Lacepede)	Misa	largemouth bass	achigan a grande bouche
Percidae	Perc	darter sp.	dard spp.
Perca flavescens (Mitchill)	Pefl	yellow perch	perchaude
Stizostedion canadense (Smith)	Stca	sauger	dore noir
Stizostedion vitreum (Mitchill)	Stvi	walleye	dore
Ethecstoma nigrum Rafinesque	Etni	johnny darter	raseux-de-terre

A.2 DESCRIPTIVE STATISTICS OF THE PARAMETERS

Count, mean and standard error of the mean (SEM) for lipid content, length and contaminant residues in young-of-the-year forage fish. sp =species: 1 = spottail shiner; 2 = yellow perch. Residues in mg/kg body content (wet weight) unless otherwise stated.

	sp	lip %	leng mm	HCB	DDE	PCB	Arochlor 1254	Arochlor 1260	Hepta-chlor	Aldrin	Hg	Cd	Cr	Cu	Ni	Pb	Zn	As	Se
det.limit ->				1.0 ug/kg	.001	.01	.01	.01	1.0 ug/kg	1.0 ug/kg	.01	.02	.2	.2	.05	.1	.2	.05	.05
station 1																			
COUNT	1	3.00	3.0	3.0	3.000	3.00	3.00	3.00	3.0	3.0	3.00	3.00	3.00	3.00	3.00	3.00	3.0	3.00	3.00
MEAN	1	2.96	39.8	0.5	0.006	0.17	0.13	0.03	0.5	0.5	0.10	0.01	0.41	0.86	0.21	0.22	46.9	0.15	0.68
SEM	1	0.13	0.7	0.0	3e-04	0.01	9e-03	3e-03	0.0	0.0	9e-03	0.00	0.13	0.02	0.12	0.03	0.6	0.00	9e-03
COUNT	2	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
station 3																			
COUNT	1	3.00	3.0	3.0	3.000	3.00	3.00	3.00	3.0	3.0	3.00	3.00	3.00	3.00	3.00	3.00	3.0	3.00	3.00
MEAN	1	2.92	46.9	4.7	0.010	0.22	0.18	0.05	0.5	0.5	0.10	0.10	0.29	0.91	0.06	0.19	46.2	0.18	0.69
SEM	1	0.09	0.7	0.3	3e-04	3e-03	3e-03	0.00	0.0	0.0	3e-03	3e-03	0.03	0.05	0.01	0.01	0.3	9e-03	6e-03
COUNT	2	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
station 4																			
COUNT	1	3.00	3.0	3.0	3.000	3.00	3.00	3.00	3.0	3.0	3.00	3.00	3.00	3.00	3.00	3.00	3.0	3.00	3.00
MEAN	1	3.02	48.1	22.0	0.009	0.17	0.13	0.04	0.5	0.5	0.22	0.01	0.38	0.75	0.09	0.13	42.2	0.10	0.80
SEM	1	0.17	0.8	0.6	3e-04	0.01	6e-03	7e-03	0.0	0.0	0.02	0.00	0.03	9e-03	0.01	3e-03	0.7	6e-03	0.03
COUNT	2	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
station 11																			
COUNT	1	3.00	3.0	3.0	3.000	3.00	3.00	3.00	3.0	3.0	3.00	3.00	3.00	3.00	3.00	3.00	3.0	3.00	3.00
MEAN	1	5.07	45.0	3.3	0.019	0.27	0.20	0.07	0.5	0.5	0.04	0.01	0.27	0.82	0.14	0.15	47.0	0.07	0.74
SEM	1	0.18	0.5	0.2	0.002	0.02	0.02	9e-03	0.0	0.0	3e-03	0.00	0.06	0.09	9e-03	0.01	1.5	0.00	3e-03
COUNT	2	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
station 12																			
COUNT	1	3.00	3.0	3.0	3.000	3.00	3.00	3.00	3.0	3.0	3.00	3.00	3.00	3.00	3.00	3.00	3.0	3.00	3.00
MEAN	1	4.42	44.3	2.3	0.017	0.29	0.21	0.08	0.5	0.5	0.05	0.04	0.42	1.07	0.33	0.15	48.2	0.13	0.75
SEM	1	0.23	0.3	0.3	0.001	0.02	0.01	9e-03	0.0	0.0	3e-03	3e-03	0.04	0.05	0.02	0.02	0.2	9e-03	1e-02
COUNT	2	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
station 13																			
COUNT	1	3.00	3.0	3.0	3.000	3.00	3.00	3.00	3.0	3.0	3.00	3.00	3.00	3.00	3.00	3.00	3.0	3.00	3.00
MEAN	1	3.30	38.5	2.7	0.012	0.22	0.17	0.05	0.5	0.5	0.04	0.01	0.18	0.72	0.17	0.12	44.8	0.08	0.73
SEM	1	0.05	0.5	0.3	0.001	0.01	0.01	3e-03	0.0	0.0	0.00	0.00	0.04	0.04	1e-02	9e-03	0.8	3e-03	0.01
COUNT	2	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00

		sp lip	leng	HCB	DDE	PCB	Arochlor 1254	Arochlor 1260	Hepta-chlor	Aldrin	Hg	Cd	Cr	Cu	Ni	Pb	Zn	As	Se
station 14																			
COUNT	1	3.00	3.0	3.0	3.000	3.00	3.00	3.00	3.0	3.0	3.00	3.00	3.00	3.00	3.00	3.00	3.0	3.00	3.00
MEAN	1	3.61	41.4	3.0	0.016	0.28	0.20	0.07	0.5	0.5	0.04	0.02	0.30	0.98	0.26	0.15	46.8	0.09	0.80
SEM	1	0.12	0.2	0.0	1e-03	3e-03	3e-03	3e-03	0.0	0.0	0.00	0.00	0.08	0.03	0.03	0.01	0.9	7e-03	7e-03
COUNT	2	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
station 15																			
COUNT	1	8.00	8.0	8.0	8.000	8.00	8.00	8.00	8.0	8.0	8.00	8.00	8.00	8.00	8.00	8.00	8.0	8.00	8.00
MEAN	1	3.60	45.3	1.4	0.010	0.20	0.15	0.05	0.5	0.5	0.04	0.01	0.89	0.82	0.69	0.14	43.2	0.15	0.78
SEM	1	0.09	0.3	0.3	3e-04	9e-03	5e-03	4e-03	0.0	0.0	1e-03	0.00	0.21	0.04	0.14	9e-03	1.0	7e-03	8e-03
COUNT	2	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
station 16																			
COUNT	1	3.00	3.0	3.0	3.000	3.00	3.00	3.00	3.0	3.0	3.00	3.00	3.00	3.00	3.00	3.00	3.0	3.00	3.00
MEAN	1	3.53	42.4	1.0	0.011	0.19	0.15	0.04	4.2	4.2	0.06	0.01	0.14	0.54	0.13	0.05	42.0	0.05	0.47
SEM	1	0.12	0.2	0.5	3e-04	0.02	0.01	6e-03	1.3	1.3	0.00	0.00	0.04	0.03	0.02	0.00	0.1	0.00	0.01
COUNT	2	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
station 21																			
COUNT	1	5.00	5.0	5.0	5.000	5.00	5.00	5.00	5.0	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00
MEAN	1	2.43	36.0	0.8	0.009	0.14	0.11	0.03	0.5	0.5	0.07	0.01	0.35	0.84	0.26	0.09	47.0	0.13	0.59
SEM	1	0.06	0.5	0.3	7e-04	5e-03	4e-03	2e-03	0.0	0.0	2e-03	4e-03	0.04	0.06	0.02	0.02	0.9	4e-03	6e-03
COUNT	2	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
station 22																			
COUNT	1	5.00	2.0	5.0	5.000	5.00	5.00	5.00	5.0	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00
MEAN	1	2.89	39.5	0.5	0.006	0.17	0.13	0.04	0.5	0.5	0.05	0.02	0.47	1.40	0.29	0.07	38.6	0.19	0.61
SEM	1	0.02	####	0.0	2e-04	5e-03	4e-03	2e-03	0.0	0.0	2e-03	2e-03	0.03	0.04	0.02	0.01	0.5	0.03	7e-03
COUNT	2	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
station 25																			
COUNT	1	5.00	5.0	5.0	5.000	5.00	5.00	5.00	5.0	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00
MEAN	1	3.93	36.9	2.0	0.013	0.19	0.14	0.05	0.5	0.5	0.03	0.01	0.29	0.71	0.17	0.14	42.9	0.06	0.66
SEM	1	0.09	0.3	0.4	4e-04	6e-03	5e-03	2e-03	0.0	0.0	2e-03	0.00	0.02	0.02	0.01	1e-02	0.6	4e-03	8e-03
COUNT	2	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
station 27 1st sampling																			
COUNT	1	5.00	5.0	5.0	5.000	5.00	5.00	5.00	5.0	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00
MEAN	1	3.44	43.8	1.8	0.013	0.20	0.15	0.05	0.5	0.5	0.03	0.01	1.61	1.37	0.76	0.45	47.4	0.12	0.57
SEM	1	0.18	0.6	0.2	5e-04	5e-03	4e-03	2e-03	0.0	0.0	2e-03	0.00	0.13	0.04	0.06	8e-03	0.3	5e-03	6e-03
COUNT	2	3.00	3.0	3.0	3.000	3.00	3.00	3.00	3.0	3.0	3.00	3.00	3.00	3.00	3.00	3.00	3.0	3.00	3.00
MEAN	2	2.53	64.1	1.3	0.009	0.13	0.10	0.02	0.5	0.5	0.05	0.01	0.48	1.07	0.30	0.05	21.0	0.07	0.33
SEM	2	0.20	1.9	0.3	0.001	0.02	0.01	3e-03	0.0	0.0	3e-03	0.00	0.03	3e-03	0.03	0.00	0.2	9e-03	0.02

	sp	lip	leng	HCB	DDE	PCB	Arochlor 1254	Arochlor 1260	Hepta-chlor	Aldrin	Hg	Cd	Cr	Cu	Ni	Pb	Zn	As	Se
station 27 2nd sampling																			
COUNT	1	5.00	5.0	5.0	5.000	5.00	5.00	5.00	5.0	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00
MEAN	1	4.49	44.7	2.6	0.012	0.15	0.11	0.04	0.5	0.5	0.03	0.01	0.37	0.65	0.18	0.11	45.1	0.07	0.58
SEM	1	0.08	0.4	0.2	9e-04	0.01	9e-03	3e-03	0.0	0.0	2e-03	0.00	0.05	9e-02	0.02	4e-03	0.7	0.00	7e-03
COUNT	2	5.00	5.0	5.0	5.000	5.00	5.00	5.00	5.0	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00
MEAN	2	2.68	67.9	1.4	0.009	0.14	0.11	0.03	0.5	0.5	0.06	0.01	0.41	0.63	0.27	0.05	20.6	0.03	0.37
SEM	2	0.12	0.8	0.2	4e-04	5e-03	3e-03	2e-03	0.0	0.0	0.01	0.00	0.02	0.04	0.06	0.00	0.6	0.00	3e-03
station 28																			
COUNT	1	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
COUNT	2	5.00	5.0	5.0	5.000	5.00	5.00	5.00	5.0	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00
MEAN	2	3.44	62.3	0.5	0.007	0.20	0.16	0.04	0.5	0.5	0.04	0.01	1.22	1.03	0.94	0.05	21.5	0.14	0.40
SEM	2	0.17	0.5	0.0	3e-04	5e-03	5e-03	0.00	0.0	0.0	0.00	4e-03	0.03	0.03	0.06	0.00	1.1	0.01	0.01
station 29																			
COUNT	1	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
COUNT	2	5.00	5.0	5.0	5.000	5.00	5.00	5.00	5.0	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00
MEAN	2	2.07	62.4	0.5	0.005	0.13	0.10	0.03	0.5	0.5	0.05	0.01	0.53	1.11	0.44	0.05	22.6	0.08	0.37
SEM	2	0.11	1.0	0.0	2e-04	7e-03	5e-03	2e-03	0.0	0.0	2e-03	0.00	0.04	0.05	0.02	0.00	0.5	4e-03	8e-03
station 30																			
COUNT	1	3.00	3.0	3.0	3.000	3.00	3.00	3.00	3.0	3.0	3.00	3.00	3.00	3.00	3.00	3.00	3.0	3.00	3.00
MEAN	1	3.32	48.2	1.0	0.013	0.24	0.12	0.06	0.5	0.5	0.04	0.01	0.14	0.66	0.12	0.05	46.5	0.21	0.56
SEM	1	0.18	0.5	0.0	3e-04	0.01	7e-03	3e-03	0.0	0.0	3e-03	0.00	0.04	0.05	0.02	0.00	1.1	0.16	0.10
COUNT	2	5.00	5.0	5.0	5.000	5.00	5.00	5.00	5.0	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00
MEAN	2	2.39	52.3	1.2	0.009	0.18	0.14	0.05	0.5	0.5	0.03	0.01	0.46	0.83	0.27	0.05	19.7	0.06	0.36
SEM	2	0.09	0.6	0.2	4e-04	1e-02	7e-03	2e-03	0.0	0.0	2e-03	0.00	0.03	0.03	0.02	0.00	0.4	3e-03	8e-03
station 31																			
COUNT	1	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
COUNT	2	5.00	5.0	5.0	5.000	5.00	5.00	5.00	5.0	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00
MEAN	2	2.44	60.8	0.5	0.006	0.10	0.08	0.02	0.5	0.5	0.03	0.01	0.56	0.72	0.24	0.05	20.5	0.05	0.31
SEM	2	0.14	1.2	0.0	3e-04	3e-03	3e-03	0.00	0.0	0.0	0.00	0.00	0.05	0.02	0.02	0.00	0.4	8e-03	0.01
station 32																			
COUNT	1	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.30	0.00
COUNT	2	5.00	5.0	5.0	5.000	5.00	5.00	5.00	5.0	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00
MEAN	2	2.23	63.6	0.8	0.004	0.06	0.05	0.01	0.5	0.5	0.04	0.01	0.56	0.91	0.33	0.05	21.4	0.06	0.27
SEM	2	0.07	1.0	0.1	2e-04	6e-03	4e-03	2e-03	0.0	0.0	4e-03	0.00	0.06	0.03	0.05	0.00	0.6	4e-03	5e-03

	sp	lip	leng	HCB	DDE	PCB	Arochlor 1254	Arochlor 1260	Hepta-chlor	Aldrin	Hg	Cd	Cr	Cu	Ni	Pb	Zn	As	Se
station 41																			
COUNT	1	4.00	4.0	4.0	4.000	4.00	4.00	4.00	4.0	4.0	4.00	4.00	4.00	4.00	4.00	4.00	4.0	4.00	4.00
MEAN	1	3.40	46.4	1.9	0.012	0.17	0.13	0.04	0.5	0.5	0.03	0.01	0.58	0.85	0.32	0.51	43.2	0.09	0.34
SEM	1	0.08	0.2	0.5	3e-04	9e-03	5e-03	4e-03	0.0	0.0	0.00	0.00	0.05	0.03	0.02	0.03	1.1	3e-03	6e-03
COUNT	2	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
station 42																			
COUNT	1	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
COUNT	2	5.00	5.0	5.0	5.000	5.00	5.00	5.00	5.0	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00
MEAN	2	2.40	66.0	0.5	0.009	0.14	0.11	0.03	0.5	0.5	0.09	0.01	0.49	0.61	0.26	0.05	20.2	0.03	0.30
SEM	2	0.06	1.4	0.0	6e-04	5e-03	5e-03	2e-03	0.0	0.0	0.00	0.00	0.05	0.02	0.05	0.00	0.4	0.00	4e-03
station 43																			
COUNT	1	5.00	5.0	5.0	5.000	5.00	5.00	5.00	5.0	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00
MEAN	1	2.58	45.2	0.3	0.005	0.07	0.05	0.02	0.5	0.5	0.04	0.03	1.44	1.08	0.53	0.23	38.2	0.15	0.30
SEM	1	0.07	0.3	0.1	2e-04	2e-03	2e-03	2e-19	0.0	0.0	2e-03	0.00	0.24	0.03	0.02	9e-03	0.8	3e-03	5e-03
COUNT	2	5.00	5.0	5.0	5.000	5.00	5.00	5.00	5.0	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00
MEAN	2	1.98	61.9	0.5	0.002	0.05	0.03	0.01	0.5	0.5	0.06	0.01	1.78	0.31	1.14	0.16	21.4	0.09	0.24
SEM	2	0.07	0.5	0.0	2e-04	4e-03	2e-03	4e-03	0.0	0.0	4e-03	2e-03	0.23	0.03	0.15	8e-03	0.4	0.00	2e-03
station 46																			
COUNT	1	0.00	0.0	0.0	0.000	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
COUNT	2	4.00	4.0	4.0	4.000	4.00	4.00	4.00	4.0	4.0	4.00	4.00	4.00	4.00	4.00	4.00	4.0	4.00	4.00
MEAN	2	4.41	58.4	0.5	0.005	0.10	0.08	0.02	0.5	0.5	0.05	0.01	0.62	0.69	0.50	0.07	19.9	0.03	0.24
SEM	2	0.15	0.9	0.0	6e-04	0.01	9e-03	5e-03	0.0	0.0	3e-03	0.00	0.12	0.05	0.14	0.02	0.7	0.00	8e-03

APPENDIX B

B.1 BACKBONE ANALYSIS

Source: Mike Keir, Great Lakes Fisheries Research Branch, Department of Fisheries and Oceans, Canada Centre for Inland Waters, Burlington (Ont.)

B.1.1 Collagen And Mineral Content

Reference: FLANAGAN, B. & G.NICHOLS Jr., 1962. Metabolic studies of bone in vitro (collagen biosynthesis by surviving bone fragments in vitro). J. of Biol. Chem. 237: 3686-3692.

Reagents:

0.1 N NaOH: 5 ml of 10 N NaOH to 500 ml with H_2O.

10% EDTA: 100 gm Disodium Ethylenedimaine-tetracetate (EDTA) up to 1 l with H_2O after adjusting pH to 7.5. Add 10 N NaOH as needed to get EDTA to dissolve. Start with less than final volume of H_2O.

1:1 ETOH-ether: 1:1 ratio by volume of abs. Ethanol (ETOH) to distilled pet. ether.

Procedure:

1. Dissect backbone; dry at 110 oC for 1-2 hrs. for small bone or overnight for for large bone. Determine wet and dry wt. to calculate % H_2O: (wet - dry)/wet.

2. Cut dry backbone in half: use the anterior portion for mineral determinations and the posterior portion for collagen determinations.

3. Mineral portion: add the appropriate amt. of 6 N HCl (depends on wt.) and heat in oven at 110 oC overnight. Determine calcium and phosphorus on this hydrolysate (may have to be diluted in a 2nd set of test tubes).

Collagen portion:

a. Add 4-5 ml of 0.1 N NaOH; shake 3-4 hrs. at room temp. for small backbone and overnight for large backbone.

b. Take backbone out of NaOH and put into 4-5 ml of 10% EDTA pH 7.5; shake overnight at 5 oC.

c. To NaOH supernatant from above add 4 ml of 10% EDTA and shake; centrifuge; white ppt. should result; discard supernatant; add 4 ml of 6 N HCl to ppt.; place in oven at 145 oC for 4 hrs.; determine hydroxyproline (see below) on this hydrolysate.

d. From part (b); aspirate off EDTA and add to bone 4-5 ml of fresh 10% EDTA pH 7.5; shake overnight at 5 oC.

e. Aspirate off EDTA and wash bone with H20; aspirate off; wash bone with acetone; aspirate off.

f. Add 4-5 ml 1:1 ETOH-ether solution to bone and shake at room temp. for 2 hrs.; aspirate off ETOH-ether.

g. Dry bone on hot sand bath plate at approx. 90-100 oC (CAUTION: do not char backbone.

h. Weigh; this is pure collagen.

i. Add appropriate amt.of 6 N HCl (depends on wt.: 1 ml 6 N HCl per mg collagen); heat in oven at 110 oC overnight or at 145 oC for 4 hrs. Determine hydroxyproline & proline after neutralization.

B.1.2 Hydroxyproline Content

Reference:

WOESSNER, J.F, Jr., 1961. The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. Arch. Biochem. Biophy. 93: 440-447.

Method:

Colorimetric. Hydroxyproline is oxidized to pyrrole-2-carboxylic acid by chloramine T which is then destroyed with perchloric acid. p-Dimethylaminobenzaldehyde is the colorimetric reagent which forms a chromogen with pyrrol-2-carboxylic acid.

Standard curve:

Make standard fresh daily. Use L-hydroxyproline diluted in H20 to make a stock standard concentration of 8 ug hydroxyproline per aliquot assayed. Assay standard curve of 8, 4, 2 and 1 ug OHPRO per aliquot used in assay step # 1.

Reagents:

0.05 M Chloramine T (sodium p- toluenesulfonchloramide):

Prepare fresh daily by dissolving 0.7 g chloramine T in 10 ml H20, adding 15 ml of methyl cellosolve (ethylene glycol monomethyl ether) and 25 ml of citrate buffer in a 50 ml volumetric flask. Keep in glass stoppered flask.

Citrate buffer:

Add 45.7 g of anhydrous citric acid, 12 ml of glacial acetic acid, 72 g anhydrous sodium acetate and 34 g of NaOH to a beaker set in ice (CAUTION: extremely exothermic reaction- prepare in hood). Add approximately 800 ml of H20 and mix. Adjust pH to 6.0. Dilute to 1 l in a volumetric flask with H20. Store in refrigerator under toluene.

3.15 M Percloric acid:

Take 27.0 ml of 70% percloric acid and dilute to 100 ml with H20.

20% p-Dimethylaminobenzaldehyde (PAB):

Stored in freezer door. Prepare shortly before use. Add approximately 25 ml methylcellosolve to a 50 ml erlenmeyer flask containing 10 g PAB. Place flask inside a beaker in a 60 oC water bath for 15-20 minutes to aid in solubilization. Dilute to volume in a 50 ml flask with cellosolve.

Procedure:

1. Place the same volume of blank (H20), standard and unknowns in clean test tubes.

2. Add 1 ml chloramine T, mix and let stand 20 minutes.

3. Add 1 ml perchloric acid, mix and let stand 5 minutes.

4. Add 1 ml PAB and mix.

5. Incubate 20 minutes in a 60 oC water bath.

6. Cool to room temperature in tap water , mix immediately prior to reading and read on spectrophotometer at 557 nm.

B.2 ANALYTICAL RESULTS

B.2.1 Backbone Analysis Of Yellow Perch

ST = station; L = length; WT = weight; COL = collagen;
VER = vertebrae (backbone); H-PRO = hydroxyproline; 1 & 2 = duplicates

ST	AGE	L. (cm)	WT. (g)	COL/ VER1 (mg/g)	COL/ VER2 (mg/g)	MEAN COL/ VER	H-PRO/ COL1 (ug/mg)	H-PRO/ COL2 (ug/mg)	MEAN H-PRO/ COL
F5	1+	11.7	21.5	297	269	283.0	44.6	38.2	41.40
F5	1+	10.4	15.9	292	297	294.5	51.4	53.5	52.45
F5	1+	11.4	19.2				32.1	32.4	32.25
F5	1+	11.8	19.9	172	141	156.5	35.7	35.3	35.50
F5	1+	12.8	26.2	108	96	102.0	30.9	38.4	34.65
F5	1+	11.8	23.1	92	21	56.5	33.1	53.5	43.30
F5	1+	12.0	23.4	285	300	292.5	46.5	43.2	44.85
SL11	1+	12.0	28.5	278	255	266.5	39.4	43.2	41.30
SL11	1+	13.0	30.0	194	234	214.0	31.7	31.7	31.70
SL11	1+	11.8	21.7	220	264	242.0	44.9	50.5	47.70
SL11	1+	12.3	28.7	296	294	295.0	48.4	40.3	44.35
SL11	1+	13.0	25.0	278	331	302.0	37.1	49.0	43.05
SL11	1+	11.5	18.4	254	268	264.0	38.1	47.7	40.40
SL11	1+	12.3	28.4	215	241	228.0	55.7	55.3	55.50
SL11	1+	12.2	21.4	255	224	239.5	50.0	55.2	52.60
SL11	1+	12.2	22.4	221	214	217.5	59.9	54.7	57.30
SL11	1+	11.9	19.4	259	344	301.5	40.8	44.2	42.50
SL11	2+	15.7	47.5	289	295	292.0	49.1	45.5	47.30
SL11	2+	15.5	44.5	277	287	282.0	45.9	45.4	45.65
SL11	2+	14.9	42.3	272	290	281.0	47.4	49.6	48.50
SL11	2+	15.0	40.3				38.2		38.20
SL11	2+	14.3	38.9	310	285	297.5	48.5	51.5	50.00
SL11	2+	14.6	45.2	337	316	326.5	25.2	25.3	25.25
SL11	2+	15.0	45.8	303	286	294.5	24.6	29.6	27.10
SL11	2+	14.6	46.9	321	311	316.0	29.1	27.7	28.40
SL11	2+	16.2	52.2	200	190	195.0	35.9	34.4	35.15
SL11	2+	15.0	48.0	206	199	202.5	51.5	40.0	45.75
SL11	Ad.	20.4	122.6	331	268	299.5	41.9	54.7	48.30
SL11	Ad.	19.7	105.5	272	265	268.5	50.5	55.7	52.60
SL11	Ad.	20.0	99.2	279	279	272.0	31.8	32.2	32.00
SL11	Ad.	19.5	93.5	290	282	280.5	57.6	57.8	57.70
SL11	Ad.	19.5	83.5	294	241	267.5	35.8	32.6	34.20

ST = station; L = length; WT = weight; COL = collagen;
VER = vertebrae (backbone); H-PRO = hydroxyproline; 1 & 2 = duplicates

ST	AGE	L. (cm)	WT. (g)	COL/ VER1 (mg/g)	COL/ VER2 (mg/g)	MEAN COL/ VER	H-PRO/ COL1 (ug/mg)	H-PRO/ COL2 (ug/mg)	MEAN H-PRO/ COL
SL5	0+	5.5	1.4	279	300	289.5	19.6	17.3	18.45
SL5	0+	6.7	3.4	319	310	314.5	24.3	22.0	23.15
SL5	0+	8.6	6.7	297	292	294.5	32.5	33.1	32.80
SL5	0+	7.0	3.2	271	253	262.0	43.2	39.1	41.15
SL5	0+	6.5	3.2	261	214	237.5	28.9	34.3	31.60
SL5	0+	8.0	5.5	213	305	259.0	45.0	35.5	40.25
SL5	0+	7.0	4.1	271	344	307.5	26.5	21.6	24.05
SL5	0+	7.0	2.8	287	292	344.5	28.6	27.5	28.05
SL5	0+	5.7	1.6	271	331	301.0	16.0	23.9	19.95
SL5	0+	7.4	4.1	225	261	243.0	25.2	37.5	31.35
SL5	0+	6.3	2.9	231	276	253.5	21.3	26.1	23.70
SL5	0+	6.9	3.3	257	224	240.5	17.8	17.2	17.50
SL5	0+	8.3	5.6	286	280	283.0	36.4	29.8	33.10
SL5	0+	7.1	3.1	306		306.0	39.2		39.20
SL5	0+	6.4	3.5	330	325	327.5	22.4	26.9	24.65
SL5	0+	7.8	4.8	282	274	278.0	26.7	23.9	25.30
SL5	0+	8.0	5.0	352	333	342.5	37.0		37.00
SL5	0+	7.0	3.2	243	379	311.0	22.8	26.8	24.80
SL5	1+	12.5	22.9	298	304	301.0	38.6		38.60
SL5	1+	10.5	11.9	264	277	270.5	28.6	32.2	30.40
SL5	1+	9.0	9.0	288	312	300.0	28.8	42.0	35.40
SL5	1+	11.5	16.9	312	273	292.5	37.8	44.3	41.05
SL5	1+	11.5	16.9	325	307	316.0	29.4	34.8	32.10
SL5	1+	10.0	10.7	317	277	297.0	27.4	32.0	29.70
SL5	1+	12.8	21.4	303	316	309.5	47.6	44.3	45.95
SL5	1+	11.2	18.2	295	329	312.0	46.6	42.6	44.60
SL5	1+	13.0	25.2	250	248	249.0	64.2	56.6	60.40
SL5	1+	13.0	24.3	218	274	246.0	40.0	48.4	44.20
SL5	1+	12.7	25.0				69.2	59.5	64.35
SL5	1+	13.4	27.3	262	259	260.0	29.4	36.6	33.00
SL5	Ad.	23.0	182.4	304	381	342.5	36.4	28.0	32.20
SL5	Ad.	19.0	100.8	231		231.0	51.6		51.60
SL5	Ad.	19.5	108.5	218		218.0	47.4		47.40
SL5	Ad.	20.4	125.7	233	358	295.5	55.3	33.2	44.25
SL5	Ad.	22.0	159.2	404	343	373.5	29.0	23.2	26.10
SL5	Ad.	15.5	42.1	254	243	248.5	54.0	56.2	55.10
SL5	Ad.	14.0	34.2	283	265	274.0	45.0	47.9	46.45
SL5	Ad.	14.7	42.7	247	224	235.5	58.8	52.8	55.80
SL5	Ad.	15.2	43.6	276	269	272.5	40.1	34.1	37.10
SL5	Ad.	15.2	38.8	264	251	257.5	49.8	54.8	52.30
SL5	Ad.	16.2	49.7	254	234	244.0	69.9	62.2	66.05
SL5	Ad.	16.0	47.8	269	272	270.5	47.9	43.8	45.85
SL5	Ad.	15.2	38.8	262	255	258.5	34.2	38.0	36.10
SL5	Ad.	14.7	41.2	306	255	280.5	57.2	55.0	56.10
SL5	Ad.	13.0	30.0	239	263	251.0	47.7	39.1	43.40